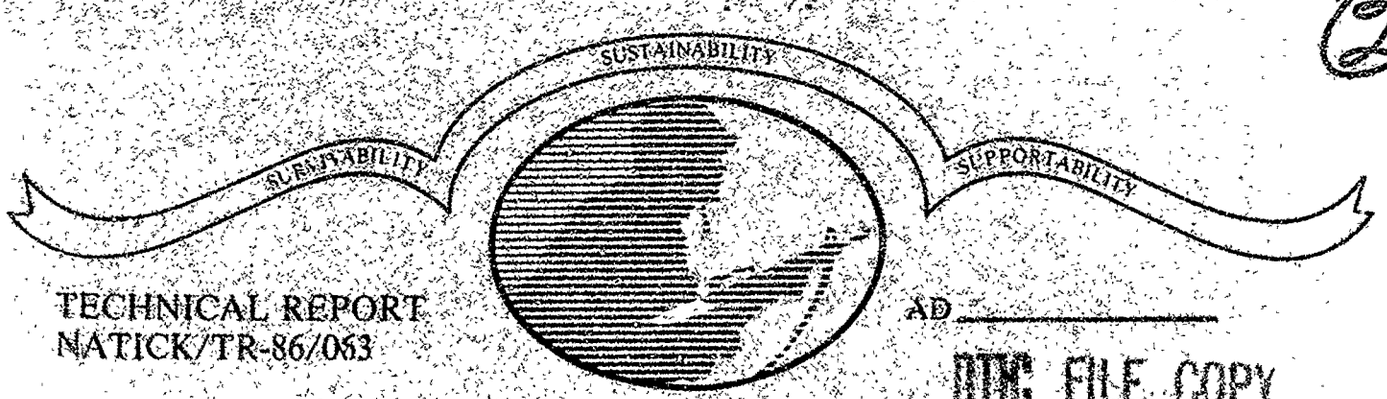

TECHNICAL REPORT
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DEVELOPMENT OF RAPID METHODS TO MONITOR OXIDATIVE AND MAILLARD (SUGAR-AMINE) POLYMERIZATION IN ENERGY-DENSE, ENCAPSULATED MODEL RATION SYSTEMS

BY

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SCIENCE AND ADVANCED TECHNOLOGY DIRECTORATE

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Energy-dense model systems containing lactose as carbohydrate, casein as protein, and soy-lecithin encapsulated corn oil as lipid have been monitored during storage at high temperatures for polymerization by means of chemical and spectrophotometric methods, including both absorption and fluorescence. The objective was to develop rapid, labor-saving methods applicable to both soluble and heavily cross-linked, insoluble components of the model systems. Systems developed to monitor Maillard browning include front-face fluorescence of acid-precipitated casein slurries, fluorescence and 410 nm absorption of the aqueous phase after pronase digestion and low intensity fluorescence of the chloroform-methanol extract of browned material. Lipid oxidation methods developed include vapor phase detection by means of polyamide plate fluorescence in presence of oxidizing lipid, front-face fluorescence, and high intensity fluorescence of the chloroform-methanol extract. The chloroform-methanol and front-face fluorescence methods in both lipid oxidation and Maillard browning and the polyamide method in lipid oxidation are relatively free from interference arising from common food components. They are potentially widely applicable and susceptible to semiautomation.

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PREFACE

The work reported here was performed under Project No. IL161102AH52Ø3Ø2Ø, Degradative Mechanisms in Dry, Moist and Fluid High Density Ration Components, Shelf Life Prediction and Extension. William L. Porter was the Project Officer and Principal Investigator. John Kapsalis had administrative supervision.

The editorial assistance of Dr. K. A. Narayan, SATD, and Mr. Norman Harris, FED, is gratefully acknowledged, as is the able assistance of Ms. Patricia Crawford in preparing and organizing the manuscript for publication.

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[1]Initials of worker, cited to show comparability of results.

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DEVELOPMENT OF RAPID METHODS TO MONITOR OXIDATIVE AND MAILLARD (SUGAR-AMINE) POLYMERIZATION IN ENERGY-DENSE, ENCAPSULATED MODEL RATION SYSTEMS

INTRODUCTION

The work reported here is methods development to study degradative mechanisms in encapsulated, energy-dense, military combat ration systems. The ultimate objective is the prediction and extension of storage life under extreme climatic and processing conditions.

The approach used was to develop encapsulated, energy-dense model systems representative of dry, moist and fluid rations and to study their quality loss due to Maillard (sugar-amine) and oxidative polymerization. It was important to develop rapid measurement methods that could monitor the two concurrent processes and could differentiate them, since they are mutually competitive and interactive.

Quality loss for this work was defined as loss of functionality and acceptance due to toughness, and loss of solubility and dispersibility. The work is not concerned with loss of nutritional quality, flavor, odor or color, although these may or may not be concomitants. The attributes studied here are largely a function of cross-linking and resultant polymerization, which it was found, are closely correlated with color and fluorescence development. The latter two characteristics are the basis of most methods derived in this report. In particular, fluorescence, when judiciously applied, can be extremely sensitive and selective, permitting differentiation between oxidation and Maillard reactions, and separation of these from test responses due to nondegraded food components.

In food storage life prediction, it is necessary to determine four things: 1) the attribute (nutrients, toughness, odor) whose loss or appearance will end storage life, giving an end point; 2) the rate of loss of that attribute as a function of temperature (and for some applications, humidity); 3) climatic or process distributions of ambient temperature in time and space, and 4) the induced temperature in the food as a result of cyclical ambient temperatures. For the purpose of this work, polymerization was chosen as the storage life limiter, and the rate of its increase as a function of changing temperature was the desired kinetic datum. Although three levels of water activity were used, quality loss as a function of water activity was not a prime target. Data on extreme induced temperatures in stored foods and the ambient climatic and processing temperatures producing these induced levels are available to this laboratory.[1]

The ration models developed for this work were energy-dense paradigms for the so-called "Nutritional Sustainment Modules" currently under design for use by forward infantry elements under extreme mobility and stress conditions anticipated in the year 2000. Energy density, whether per unit mass or volume, implies dried, and, for volume density, compressed items. Beyond that for further densification, the substitution of lipid for portions of protein or carbohydrate is mandatory. Under current preventive medical protocols, a substantial fraction of polyunsaturated lipid is recommended, which, in turn, means relatively fluid and oxidizable material. Such lipid often requires encapsulation, to permit water miscibility and prevent "oiling out". In addition, phospholipid encapsulation (Fig 1) in conjunction with competent primary antioxidants,

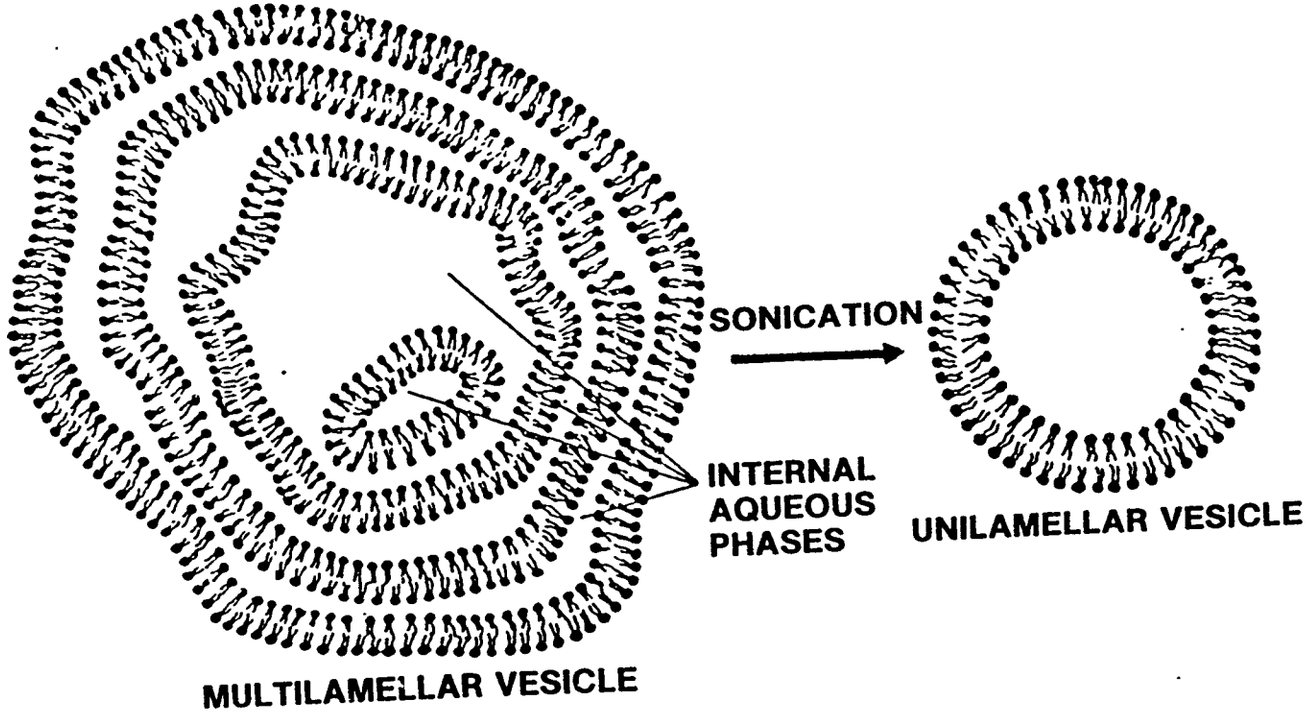

Figure 1. Sonicated phospholipid vesicles.

may reduce oxidative degradation, a primary cause of cross-linking. Typical naturally occurring encapsulated energy-dense foods are soybeans, egg yolk, and cream. Mayonnaise and salad dressing typify fabricated foods using egg phospholipid encapsulants. The latter together with soy and milk phospholipids share certain important advantages: 1) they are GRAS, or generally regarded as safe as food components or adjuvants by the Food and Drug Administration (FDA), 2) they are excellent encapsulants and emulsifiers, and 3) as stated above, they are strong synergists with antioxidants. These components have been used exclusively in the work described in this report and became the basis of two important measurement methods.

METHODS DEVELOPMENT

For food storage life prediction, there was a need for methods to detect cross-linking and polymerization due either to sugar-amine (Maillard)browning or to lipid oxidation (Fig. 2). It was desired that the methods be rapid and labor-saving, so that real time measurement of the level of quality loss could be made. It was also desirable that the methods differentiate the two modes of loss, especially when both are occurring concurrently.

A. Past Methods

Previous workers have developed general measurement methods for sugar-amine browning and oxidative degradation. The early work was targetted largely toward nutritional availability. Many of the standard methods measure the first stable products formed, i.e., the Amadori compound, a 1-amino-1-deoxy-2-ketose compound, or lipid hydroperoxide. Both are colorless, relatively bland in taste, odorless and do not involve

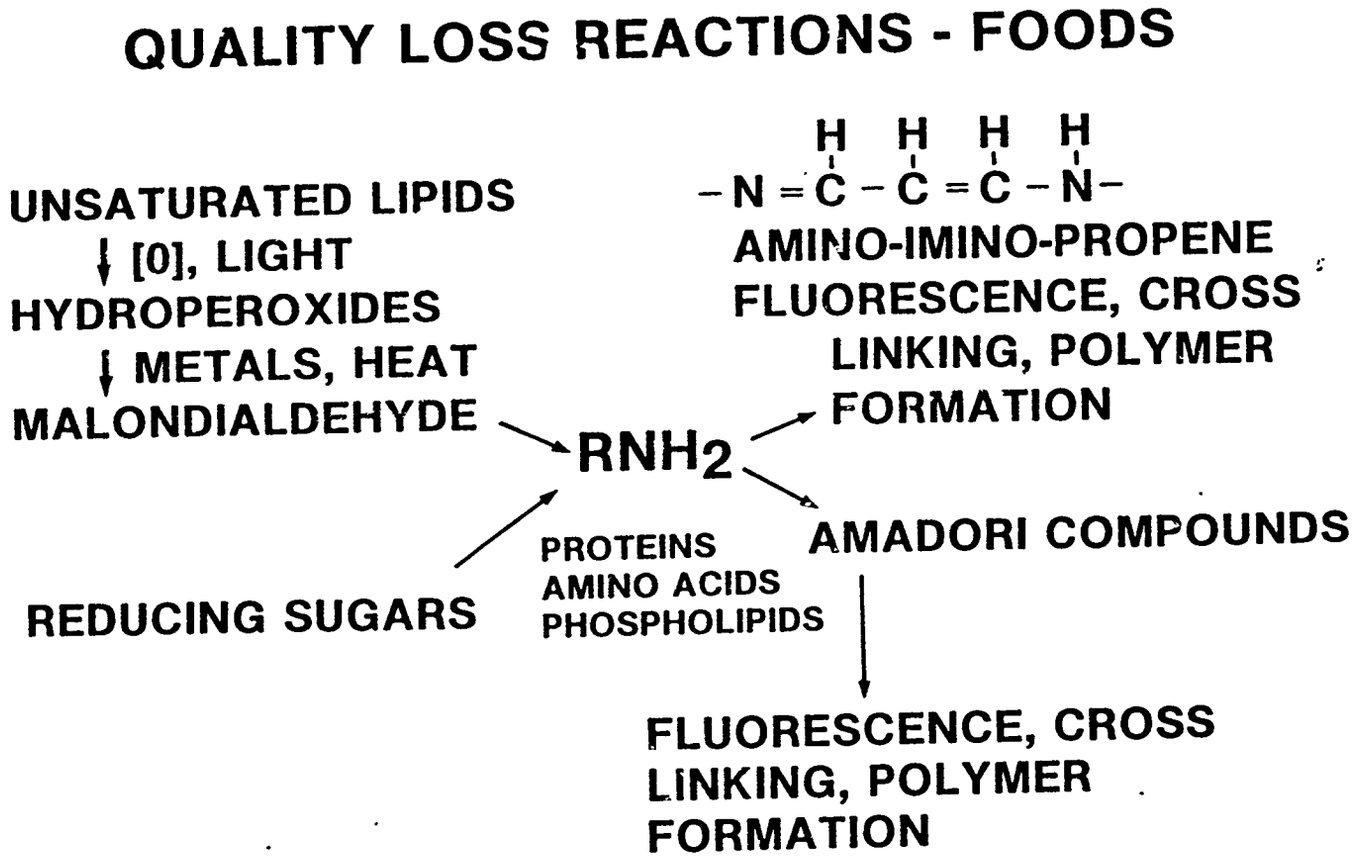

Figure 2. Quality loss reactions - foods.

polymerization. However, both render the ingredients, sugar and amino acid or lipid, unavailable biologically. Hence, the early work was nutrition centered. Such methods include the reducing power of the Amadori compound assayed by acid-ferricyanide,[2] the formation of the artifact furosine from Amadori compounds after hot acid hydrolysis of a protein as assayed on an amino acid analyzer[3] and the iodometric determination of peroxide value.[4] The thiobarbituric acid test (TBA) has been considered to measure the dicarbonyl, malondialdehyde, from lipid oxidation, a secondary product which is a colorless, relatively odorless, and unstable monomer.[5]

The above methods are adequate for nutritional purposes and at initial stages when products are monomeric and often water-soluble. They are, however, in general, labor intensive and relatively slow. There is also interference by common food ingredients. In addition, at the stage of most interest to us, polymerization and cross-linking, those processes most closely connected to chewiness and lubriciousness, the above mentioned methods begin to fail because of the insolubility of the very products that have to be to measured. A search was made, therefore, for rapid, reproducible, labor-saving assay methods for sugar-amine browning and lipid oxidation targetted at polymeric products or products known to be correlated with polymerization.

The method developed by Kim and Taub[6] of colorimetric assay after enzymatic digestion was modified for fluorescence assay. The chloroform-methanol extraction method of Bouzas, Kamerei and Karel,[7,8,9] which was developed for study of fluorescent chromophores resulting from lipid oxidation in the presence of protein, was modified to study Maillard reaction fluorescent chromophores.

B. Our Methods

1. The Energy-dense, Encapsulated Model System.

As an idealized pattern for the many kinds of energy-dense, encapsulated combat rations which may be developed, the following model system (Table 1) was developed, primarily to permit methods development in quality loss:

TABLE 1. Encapsulated, Energy-dense Model Ration System

Component	Percentage
Stripped corn oil	35
Stripped soy lecithin	1.5
Sodium caseinate	15
Lactose	35
Starch	5
Assorted additives,* as required, including wheat bran, cocoa non-fat solids, baker's yeast	8.5

*Not used in the work reported herein.

Both the triglyceride and the lecithin phosphatides (which included the usual phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl serine as well as others) were largely, but not completely, stripped of natural antioxidants during manufacture, the

triglycerides by vacuum distillation, the phosphatides by acetone extraction. As constituted and without additives, the system has 40 weight percent lipid and about 60 percent of calories derived from lipid. It is purposely nearly depleted of natural antioxidants in order to compare concurrent browning and oxidation. The usual ration would have much more delayed oxidation, and if sucrose were substituted for lactose, much more delayed Maillard browning.

For methods development in Maillard browning, this system was stored in three moisture modes, a_w 0.23, 0.5, 1.0 (fluid) and at controlled temperatures close to 80, 90 and 100°C. The storage for lipid oxidation methods development was done at the three moisture levels and at 100°C.

Stripped corn oil was procured from Eastman Kodak Co., Rochester, N.Y. Most but not all, of the tocopherols have been removed by molecular distillation (10 ppm maximum). Sodium caseinate was Savortone(R)* Low Flavor, obtained from Express Foods Company, Louisville, KY. Soy lecithin was Arlec(R)* (acetone-stripped granules, which we have shown to contain very little natural antioxidant) obtained from Archer Daniels Midland Co. Lactose was Baker's C. P. Analyzed, J. T. Baker Chemical Co. All were used as received without further purification. The lactose (49 g) and starch (7 g) were boiled for one minute in 937 mL deionized water, cooled, and the caseinate (21 g) added and blended with a Waring blender, after which the lecithin (2.1 g) was blended. Next 49 g stripped corn oil was added drop by drop, mixing with a magnetic stirrer. The dispersion was freeze-dried for 24 hours and stored as a white powder in the freezer.

2. Sugar-amine Browning.

a. Enzymatic digest--absorption and fluorescence spectrophotometry. Using a modification of the method of Kim and Taub,[6] 100 mg of a relatively dry sample of the model system which has been heated at a suitable water activity to accomplish Maillard browning is comminuted in a mortar and added to 4 mL (0.1M) ammonium bicarbonate solution, pH 7.8, containing 2 mg Pronase(R)* (Calbiochem, Inc.) contained in a centrifuge tube. The tube is stoppered and kept in a 37°C water bath (±3°C) for 24 hours. Two mL of chloroform are added, the tube is mixed 1 min on a Vortex mixer*, and centrifuged for 1 h on a clinical centrifuge. The aqueous supernatant is used for absorption spectrophotometry at 420 nm without dilution and is appropriately diluted for fluorescence spectrophotometry using excitation at 392 nm. If turbidity develops, samples are filtered through a 0.22 µm Millex-GV* or 0.45 µm Millex-HV* filter unit (Millipore Corp.) to produce a clear solution.

With suitable dilution to avoid fluorescence quenching, this method gives good sensitivity and signal--to--noise ratio for both fluorescence and absorption spectrophotometry, the latter being at least 10 times less sensitive. The method failed when applied to samples heated at lower water activities (a_w 0.23, saturated potassium acetate solution) because of unfilterable turbidity, the precipitate being fluorescent and colored. The method is slow and labor-intensive.

Fluorescence is measured on a Baird-Atomic Fluorescence Spectrophotometer, Model SF-1*. Absorption is measured on a Cary Model 15* Spectrophotometer.

Typical settings for the Baird Fluorescence Spectrophotometer are: Coarse gain 10, fine gain 6, entrance slit fine, exit slit medium, excitation wavelength 392 nm. All readings are expressed as the ratio 100 x Fluorescence Intensity of Sample/Fluorescence Intensity of Quinine Sulphate (1 ppm in 0.1 N H_2SO_4).

b. Chloroform-methanol extract (C/M). Using a modification of the method of Bouzas, Kamerei and Karel[7,8,9] a measured 100 mg of a relatively dry sample which has been heated at a suitable water activity to accomplish Maillard browning is comminuted in an agate mortar and added to a separatory funnel containing 4 mL of deionized water and 20 mL of a chloroform-methanol solution (2/1, v/v, spectral grade, Burdick and Jackson, Inc.) the water and organic solvent being left initially in two layers. The separatory funnel is stoppered and shaken a timed 5 minutes. The contents are decanted into a centrifuge tube without washing and centrifuged on a clinical centrifuge for a timed 5 minutes. Circa 4 mL of the clear lower layer (92% $CHCl_3$/8% MEOH) are carefully transferred by a 9" Pasteur disposable pipette into a 5 mL test tube, stoppered with aluminum foil. A measured 100 mg of anhydrous sodium sulfate is added and stirred by careful plunger action of a glass rod. An initial colloidal opalescence usually clears immediately and the fluorescence of the solution is measured on a Baird-Atomic Fluorescence Spectrophotometer at excitation wavelength of 380 nm. Emission is in the 450-460 nm range. Compartment slits are at medium and coarse gain is 100, fine gain 9. This method is about ten times as fast as the enzymatic method, is reproducible and much less labor intensive, especially when turbidity is a problem, as mentioned above. It is applicable in all

cases, even those for which turbidity renders the enzymatic method unsuitable. The only interfering compound of importance in biological systems is retinol, vitamin A, which can be destroyed by brief irradiation at 360 nm. The method has recently been successfully automated using the Soxtec System HT*, an accelerated Soxhlet extraction method permitting analysis of six samples in two hours.

c. Front-face fluorescence of acid-precipitated casein slurry. To five mL of a suitably browned fluid sample (100°C, 2.5 hours), containing 2.43 g dry weight, is added 20 mL deionized water. The dispersion is acidified to pH 4.6 with 1N HCl, with stirring. The precipitate is centrifuged and washed with 10 mL deionized water three times. By pressing a 9 mm thick quartz wedge into the 10 mm silica cell containing 0.5 mL of a 15 percent slurry of the precipitate in water, a 1 mm thick slab of slurry is forced up into the void space. The slurry shows little to no settling and on the Baird-Atomic Fluorescence Spectrophotometer, using excitation at 360 nm and front-face viewing at an inclination of 52°C from normal to the cell face, produces reproducible fluorescence emission at 445 nm. It was demonstrated that any riboflavin interference is removed by the water washings of the acid-precipitated protein. Table 2 gives values of the fluorescence index at selected heating times for a heat-browned fluid nonfat dry milk. The index is a normalizing procedure to create an internal reference by dividing fluorescence intensity at the emission maximum by intensity at the minimum between the scattered 360 nm peak and the fluorescence emission peak. In scattering samples like these, the fluorescence index so derived

TABLE 2. Slurry Fluorescence of Acid-precipitated Casein from Heat-browned Fluid Nonfat Dry Milk (100°C, 2.5 h)

Time (min)	Fluorescence Index
0	1.22
30	1.34
60	1.93
90	2.32
120	2.88
150	3.05

is a dependable normalization method when only event marking (induction period) or relative rates are required.

Alternatively, front-face slurry fluorescence is measured on a Spex Fluorolog Spectrophotometer* at an angle of 22°. Excitation is at 360 nm, typical emission 438-445 nm, slit-widths, ex 2.0 mm, em 1.0 mm with a 1 nm increment and integration time of 0.1 second. The instrument is in the DC mode. For this instrument, a 390 nm cut-off filter is placed in the emission path, and the spectrum is scanned through the residual of the excitation scatter peak at 360 nm. The latter is then used as the normalizing reference for the fluorescence index, instead of the minimum.

3. Lipid Oxidation.

a. Chloroform-methanol extract (C/M). It has been found by us and others[7,8,9] that the standard method described above (Para II, B., 2-b) can be used also to monitor lipid oxidation. Oxidation was found in dry samples which were browned at 100°C and low water activity (a_w 0.23,

saturated potassium acetate solution), for periods of 24 hours or longer, at which time oxidation is known to commence, as is revealed by vapor phase methods (see below, Para II, B., 3-b). Fluorescence measurements (Baird-Atomic instrument) on the C/M extract using an excitation wavelength of 380 nm, as is used for Maillard browning measurements, in the presence of lipid oxidation begin to show a greatly enhanced fluorescence to color intensity ratio. This intense emission at low color levels (highlightness--Hunter L) is highly atypical for Maillard browning and can be used to measure lipid oxidation, since its magnitude dwarfs the Maillard contribution. Work is currently underway to differentiate more sharply the Maillard from the oxidative contribution to the C/M extracts.

b. Front-face fluorescence of acid-precipitated casein slurry. It was found that, as in the chloroform-methanol method, front-face fluorescence is a very sensitive method to measure extent of oxidative polymerization as well as Maillard reaction by means of fluorescence of the amino-imino-propene product from lipid carbonyl reactions with protein lysine. Emission can be distinguished from Maillard fluorescence emission by the high values at high lightness (Hunter L) and by the independent confirmation provided by oxidative polyamide fluorescence or 232 nm absorption of a hexane extract.

c. Oxidative polyamide fluorescence (OPF). Porter et al.[10] showed that the vapors from oxidizing lipids (whether triglyceride, polar lipid, or simple esters or acids) when in contact with polyamide powders, produce characteristic fluorescence, similar to that shown for amino-imino-propene compounds by Chio and Tappel.[11] Solid sample methods for measuring this

fluorescence and many examples of its use, have been reported by Porter.[10]

The method was applied to the present energy-dense, encapsulated model systems in three modes, dry, moist and fluid. In all cases, terephthalate plates measuring 2 x 3 cm and coated with a 250μ polyamide layer were suspended powder face down on aluminum mesh screen over open 5 cm petri dish bottoms containing a carefully weighed 300 mg of dry model system comminuted powder. The petri dish, in turn, was supported on an overturned 25 mL beaker standing in a half-pint Ball Corp. mason jar containing approximately 20 mL of either saturated potassium acetate, (a_w 0.23) or saturated sodium bromide (a_w 0.5). The screw-top lid of the Ball Corp. mason jar was carefully lined with aluminum foil, since it has been found that the rubber seal ring contributes oxidative fluorescence. The jars were equilibrated overnight in the dark at room temperature after sealing, and were placed in a 100°C draft oven for various storage times. Polyamide plates were read on a solid sample holder in a Baird-Atomic Fluorescence Spectrometer, using excitation wavelength
360 nm. Coarse gain is 10, fine gain 0, with entrance slit fine, exit slit medium. Emission begins at 425 nm and undergoes a steady red shift as oxidation proceeds, which is a characteristic of oxidative but not Maillard fluorescence. A jar containing all elements except the model system is heated as a blank control and no increase in emission over that of blank occurs during Maillard browning alone.

4. Reflectance Colorimetry.

Color values, expressed as Hunter L, a, b, were measured and computed on a Hunter lab D54P-5 Reflectance Spectrophotometer*. The instrument was

suitably standardized with a light trap, white standard and gray standard, with specular component included.

RESULTS AND DISCUSSION

The detailed use of the methods developed here awaits longer and more extensive kinetic studies of quality loss. However, the results obtained while developing the methods are reported below.

A. Sugar-Amine Browning

1. Enzymatic Digest--Absorption and Fluorescence Spectrophotometry.

Figures 3, 4, and 5 show the progress of Maillard browning during storage of the model system at 89°C and water activity 0.5 (saturated sodium bromide). Compared with 420 nm absorption, fluorescence shows a higher ratio of browned sample value to control value, is more sensitive and gives an earlier indication of the onset of polymerization. Previous work with gel filtration showed that both fluorescence and 420 nm absorption correlate with each other and with the appearance of high molecular weight material, travelling near the void volume in certain gels. The reproducibility of the enzymatic method can be assessed by comparing Figures 3 and 4, which depict two separate browning experiments by the same worker. Figure 5 shows browning measurements under the same conditions by another worker, the double sets of points representing repeats of the enzymatic digest and measurements.

Figures 6 and 7 show results using the same method, but for browning conducted at 100°C and a_w 0.5, by two different workers. Conspicuous at the higher temperature is the greater lag until onset of rapid phase

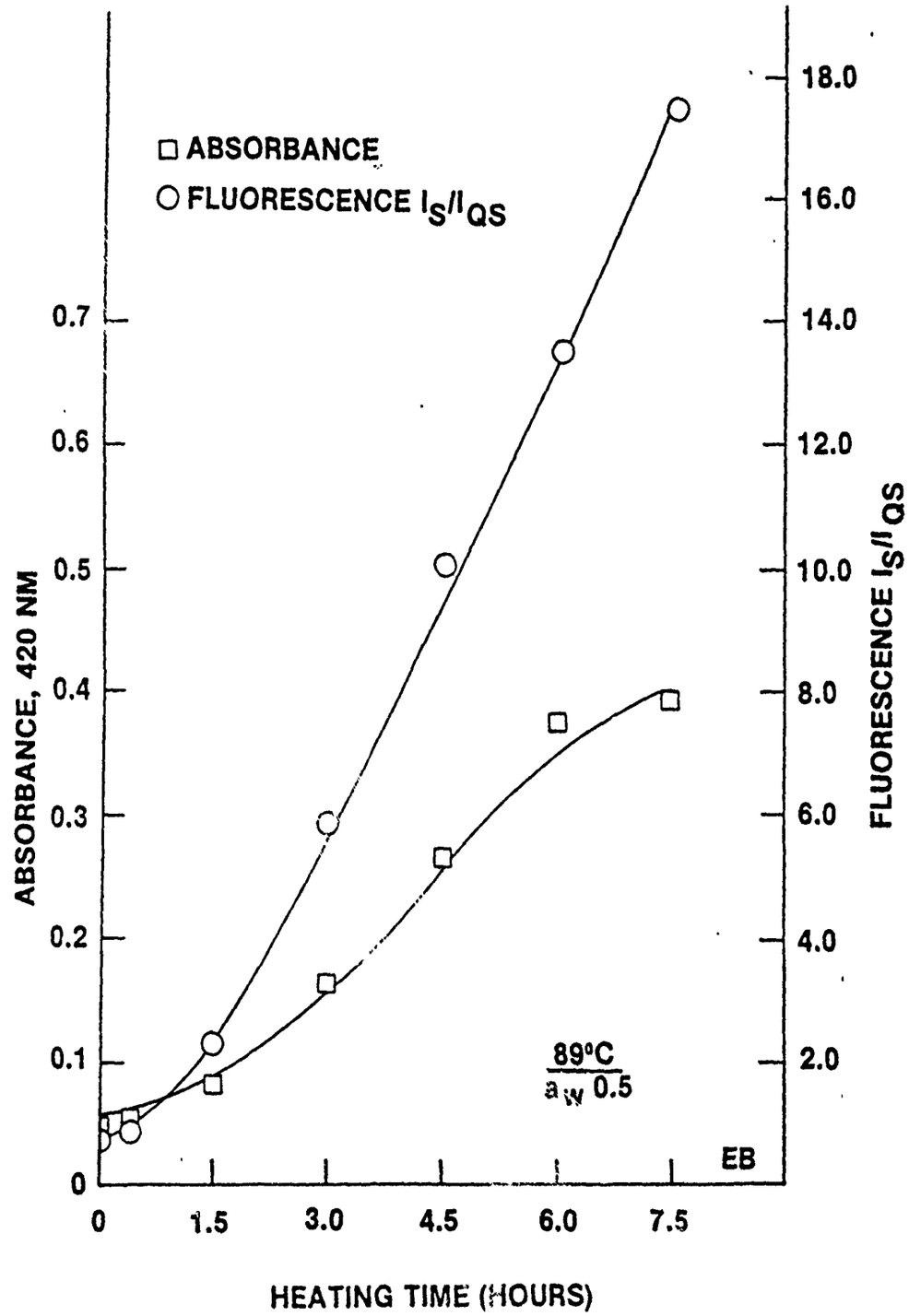

Figure 3. Maillard browning and cross-linking measured by aqueous fluorescence and 420 NM absorbance. 89°C, Aw 0.5. E.B.

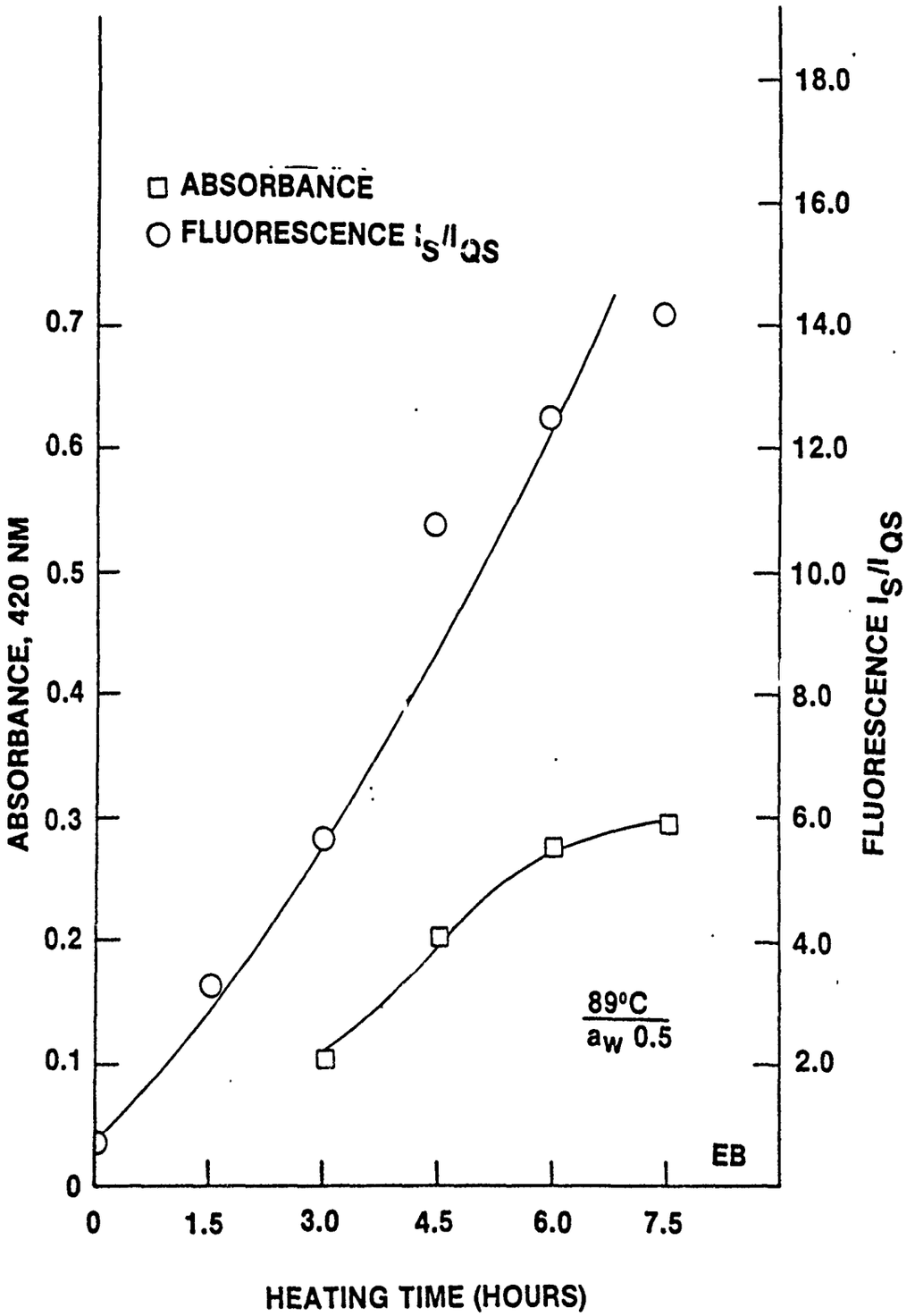

Figure 4. Maillard browning and cross-linking measured by aqueous fluorescence and 420 Nm absorbance. 89°C, Aw 0.5 E.B. Repeat

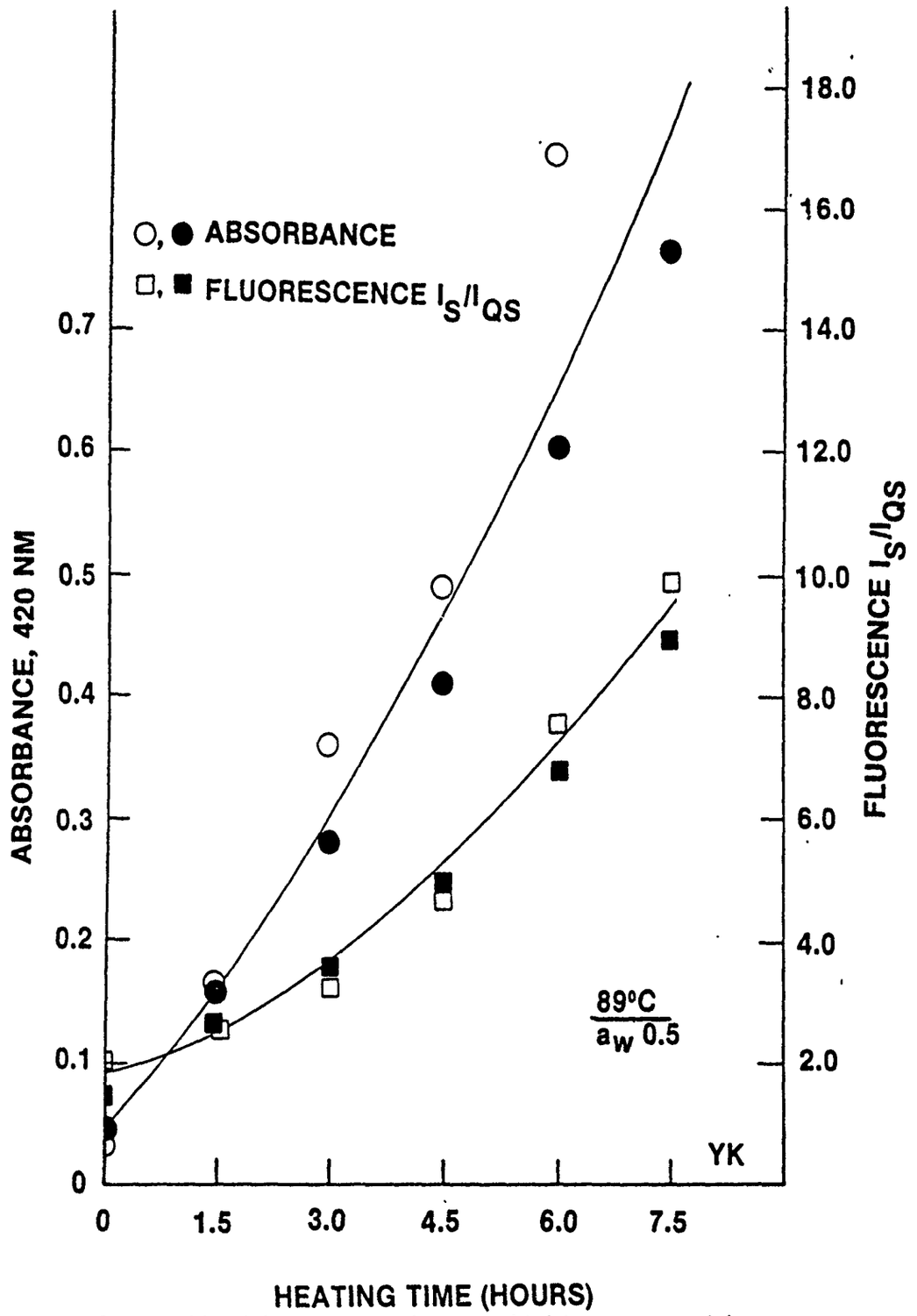

Figure 5. Maillard browning and cross-linking measured by aqueous fluorescence and 420 Nm absorbance. 89°C, Aw 0.5. Y.K.

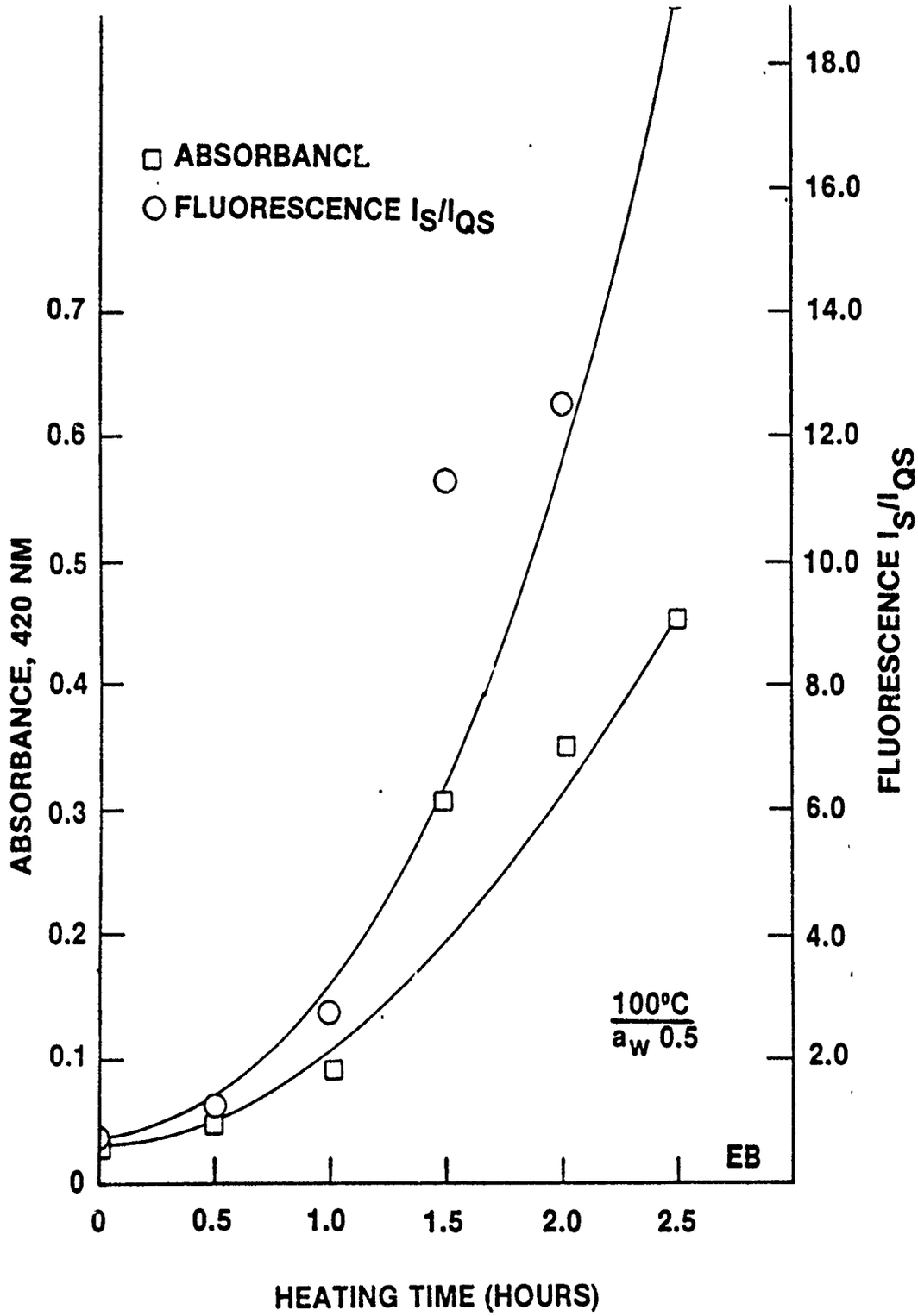

Figure 6. Maillard browning and cross-linking measured by aqueous fluorescence and 420 Nm absorbance. 100°C, Aw 0.5. E.B.

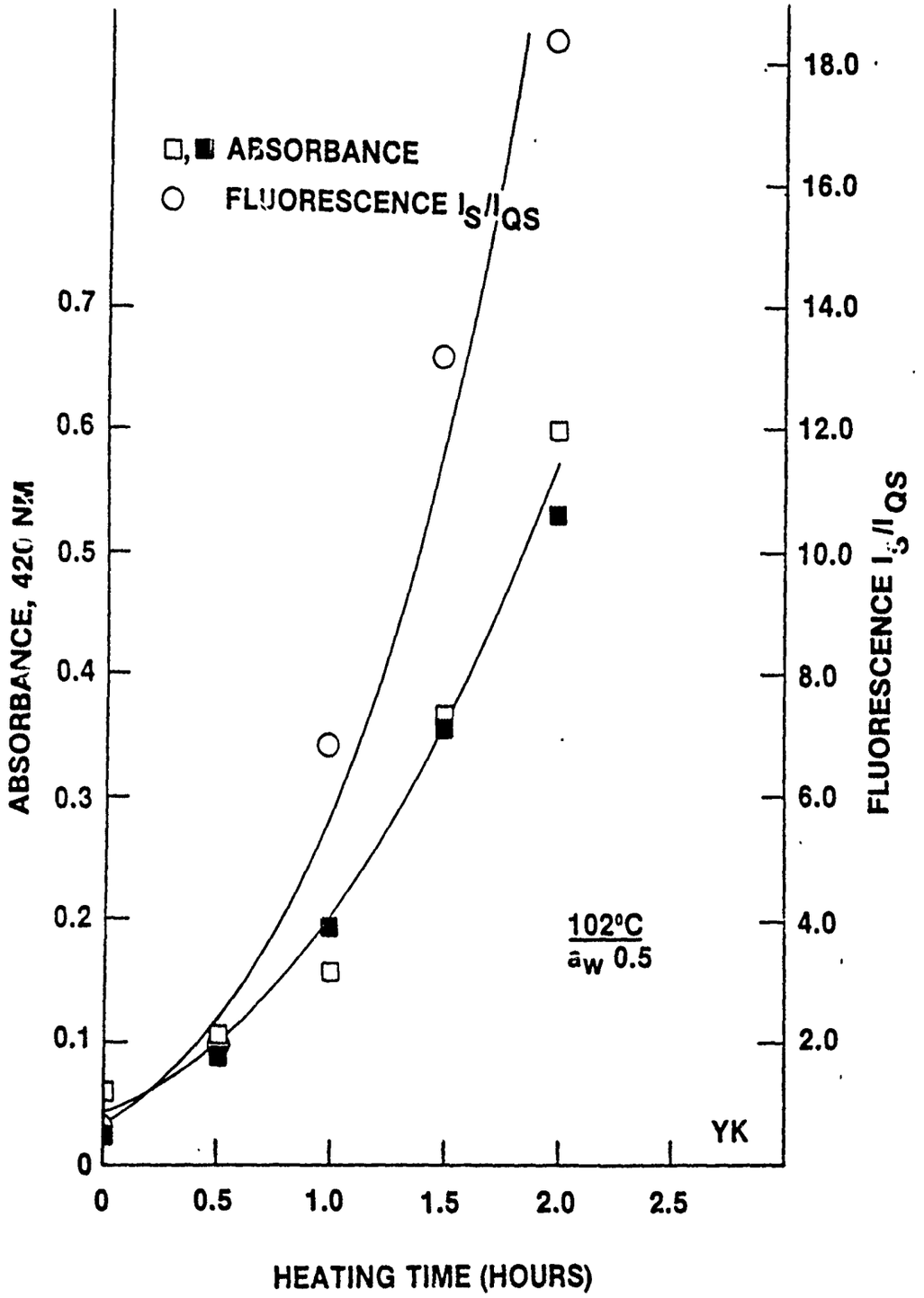

Figure 7. Maillard browning and cross-linking measured by aqueous fluorescence and 420 Nm absorbance. 102°C, Aw 0.5. Y.K.

browning, whether assayed by fluorescence or color, found by both workers.** Again, fluorescence is an earlier and more sensitive indicator.

2. Chloroform/Methanol Extract.

Figures 8, 9, and 10 show C/M measurements of the same browned samples which had been assayed enzymatically above, which were stored at 89°C and a_w 0.5. Results from the two samples heated by the same worker agree well with those of the second worker and show roughly typical Maillard kinetics (first order asymptotic) with little initial lag. The ratio of browned sample value to control value is much less than for the enzymatic digest fluorescence, but is very reproducible.

Results for the C/M extract method on the sample browned at 100°C and a_w 0.5 (Figure 11) show the same lag as those from the enzymatic digest method (Figure 6). The C/M method also duplicates the high anomaly of the 1.5 hour sample, giving evidence that this is a browning anomaly and not a measurement error.

**A careful check of internal temperatures in the reaction vessels indicates a 30-40 min lag in reaching 100°C, from initial room temperature. Subsequent readings are not affected by this lag, since jars and contents are hot.

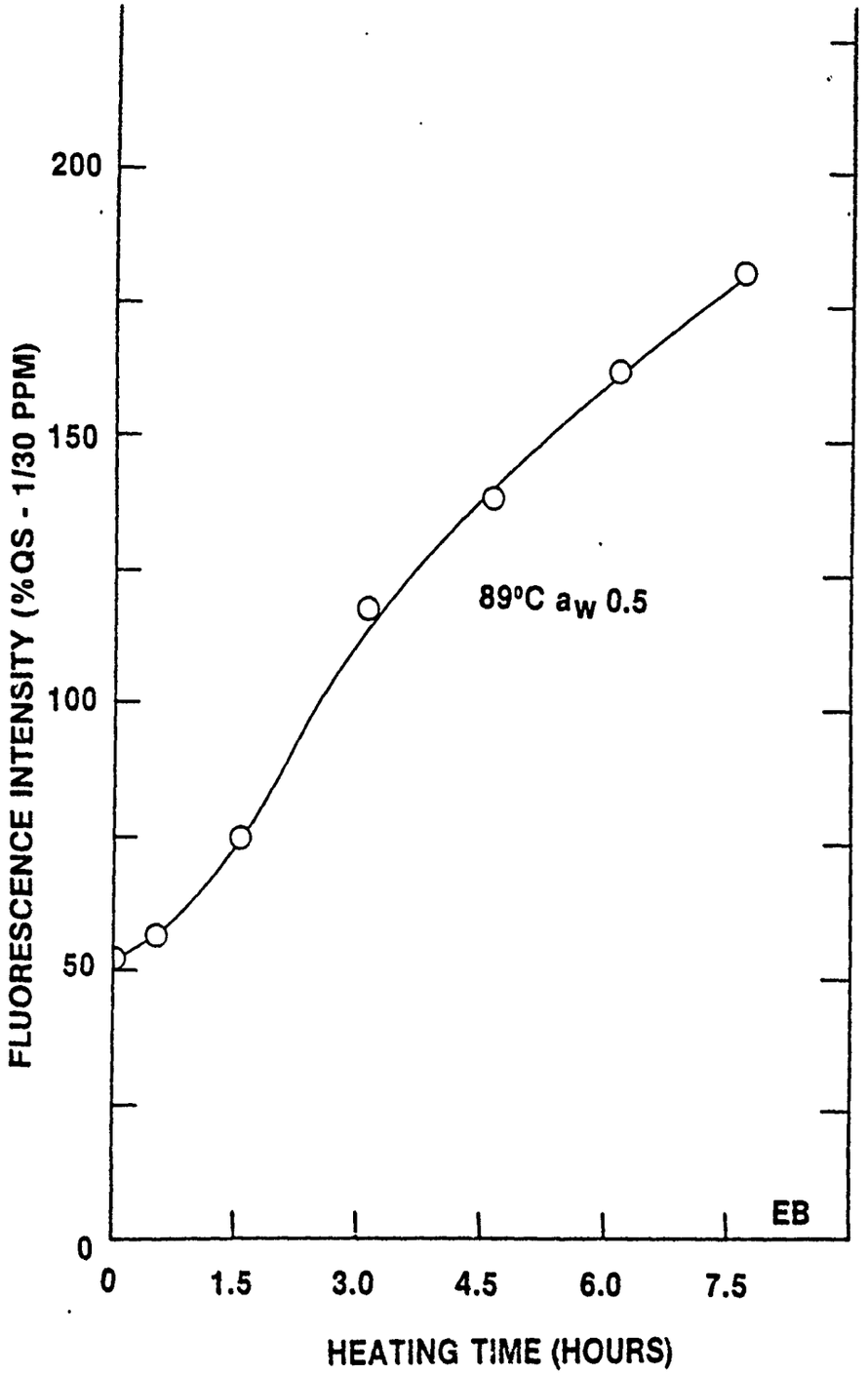

Figure 8. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 89°C, Aw 0.5. E.B.

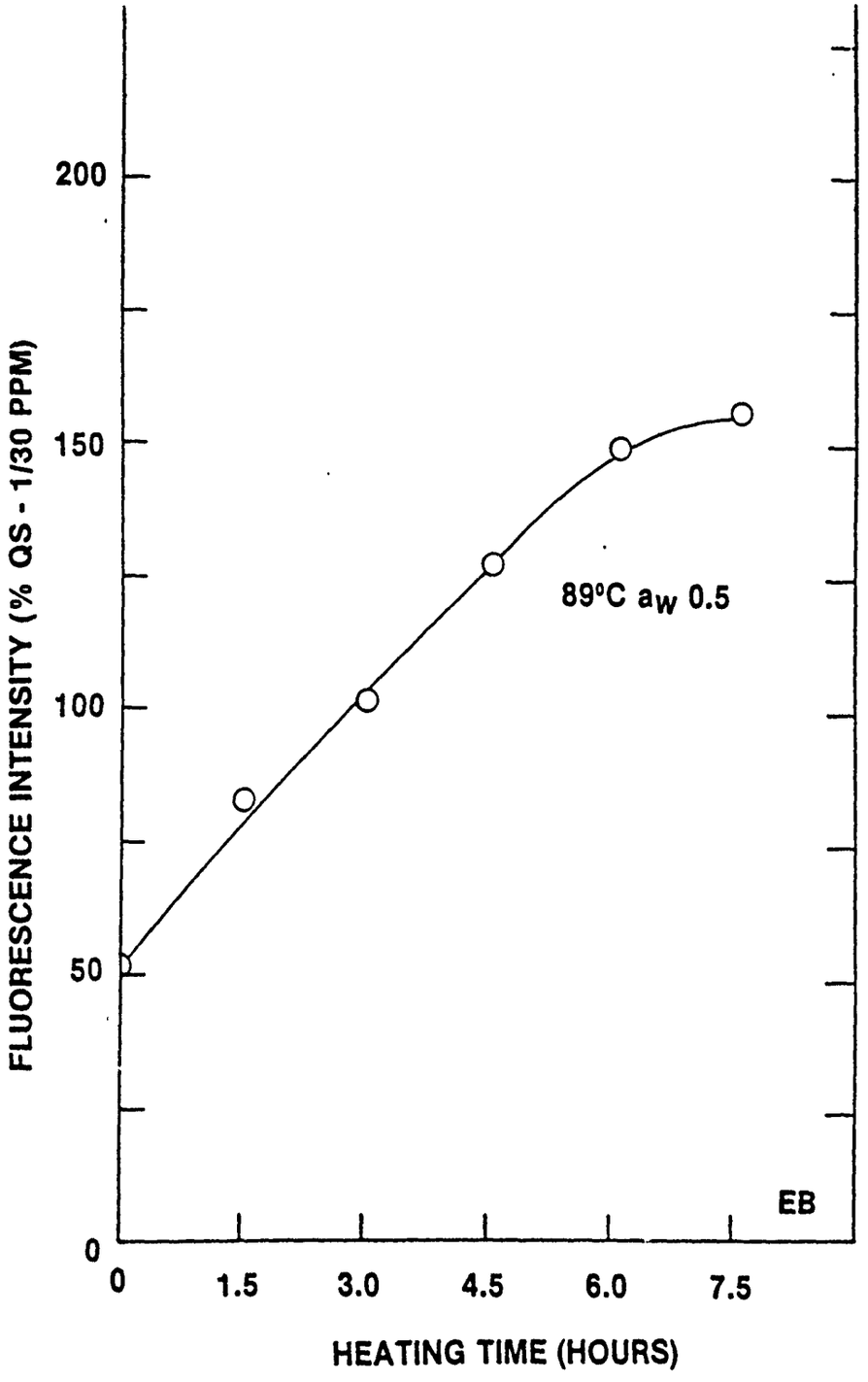

Figure 9. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 89°C, Aw 0.5. E.B. Repeat

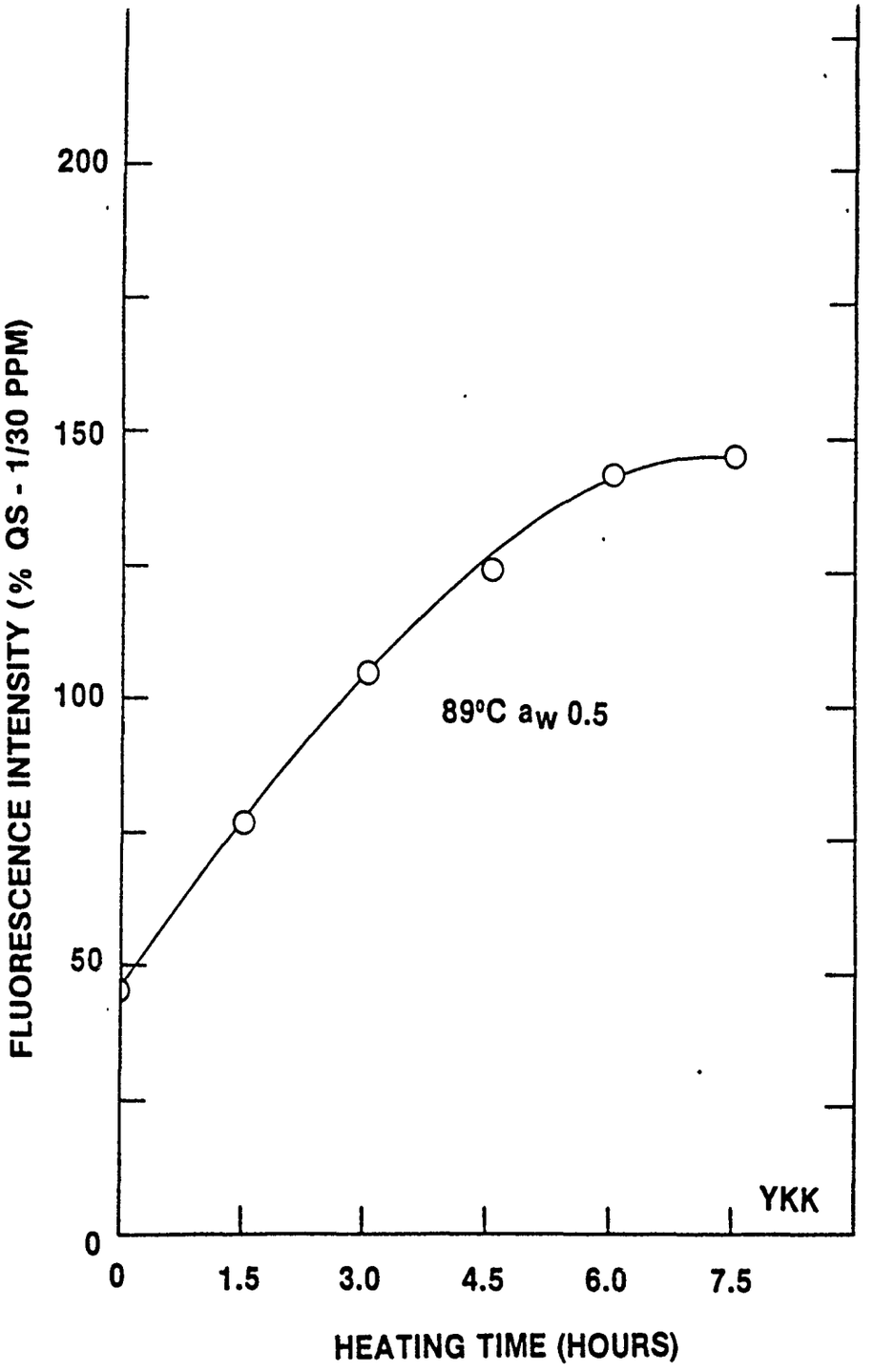

Figure 10. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 89°C, Aw 0.5. Y.K.

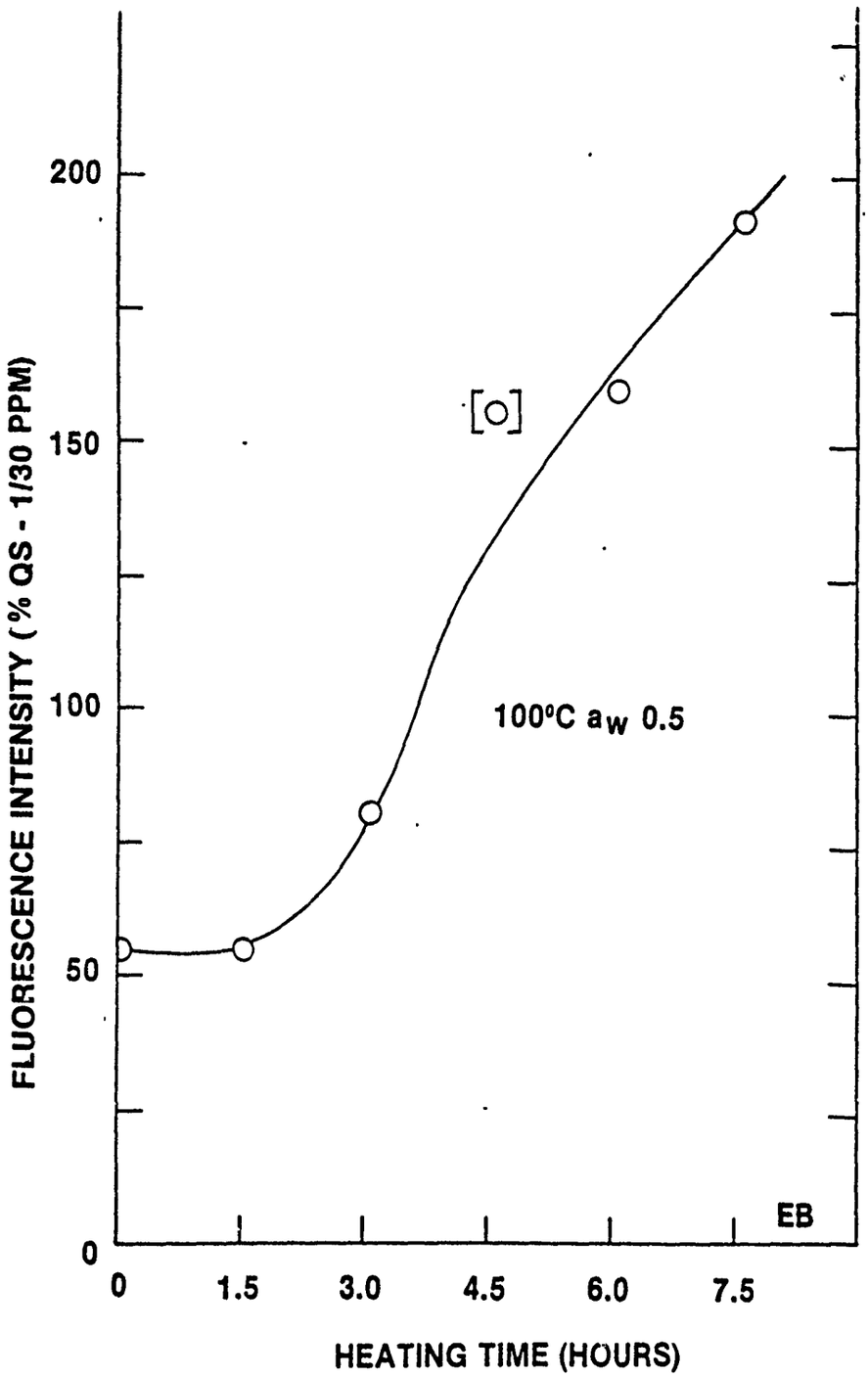

Figure 11. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 100°C, Aw 0.5. E.B.

When the C/M method was employed on samples browned at a_w Ø.23 (saturated potassium acetate), it proved far superior to the enzymatic method, which failed because of intractable turbidity in the final solutions. Figures 12 and 13 show results of C/M analysis of two sets of the drier samples browned at 1Ø2°C by the same worker. Figure 12 shows a slightly greater initial lag, but the values for the first four storage times are quite reproducible and show normal Maillard kinetics comparable to results with the moist samples. However, the fifth sample for each shows anomalously intense fluorescence at relatively high lightness (high Hunter L) of the powder, particularly in Figure 13, where the fifth sample was stored 24 hours. The product of fluorescence intensity and Hunter lightness (C/M x L in Table 3) of the latter is anomalously high, and it is accompanied by a wavelength shift. These data, coupled with observations made by the polyamide fluorescence method, which in vapor phase is specific for oxidation, suggest that the intense fluorescence at high lightness in the C/M extract is due to amino-imino-propene phospholipid products, possibly stemming from malonaldehyde, but correlated much more with oxidation than with Maillard reactions.[7,8,9]

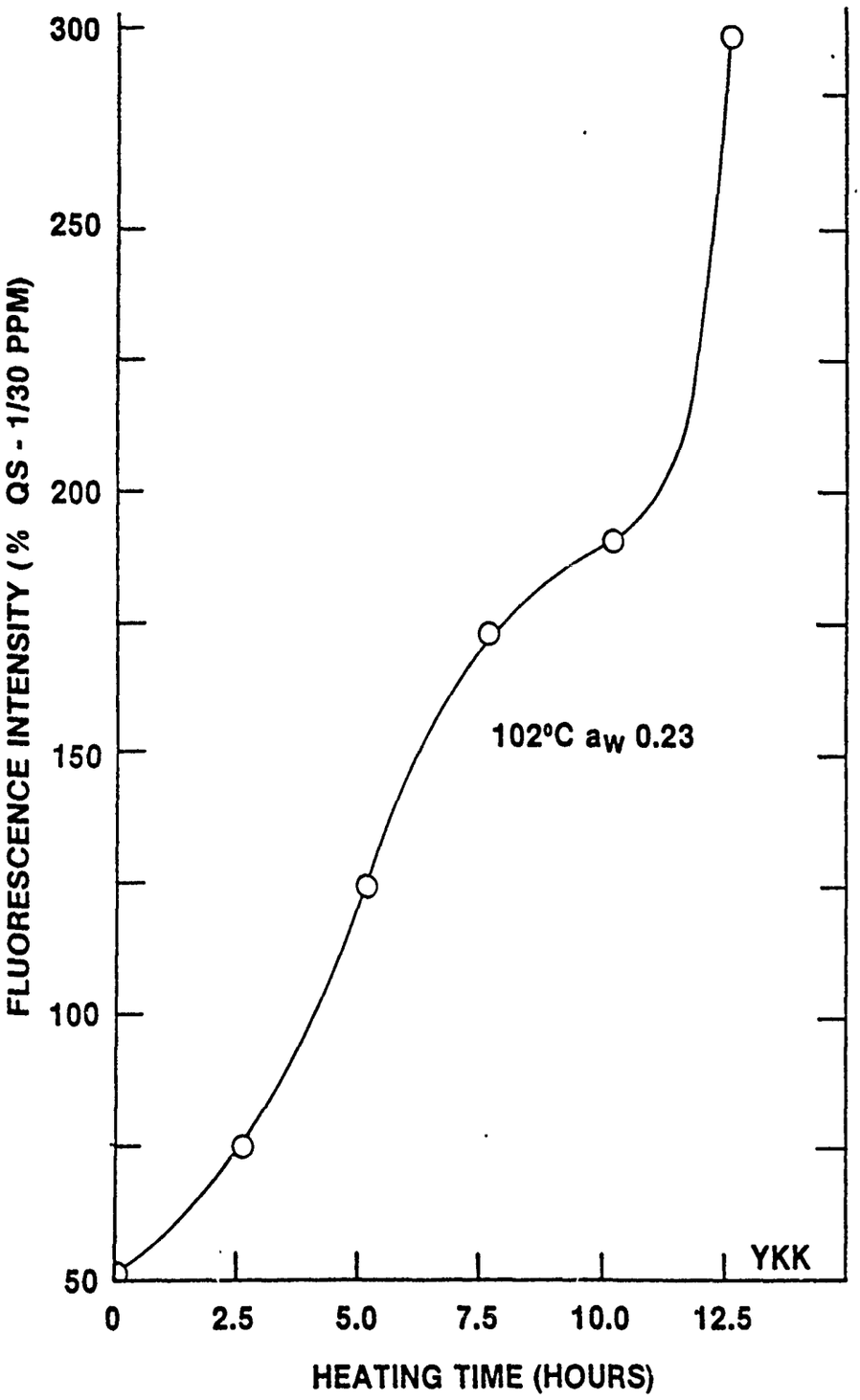

Figure 12. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 102°C, Aw 0.23. Y.K.

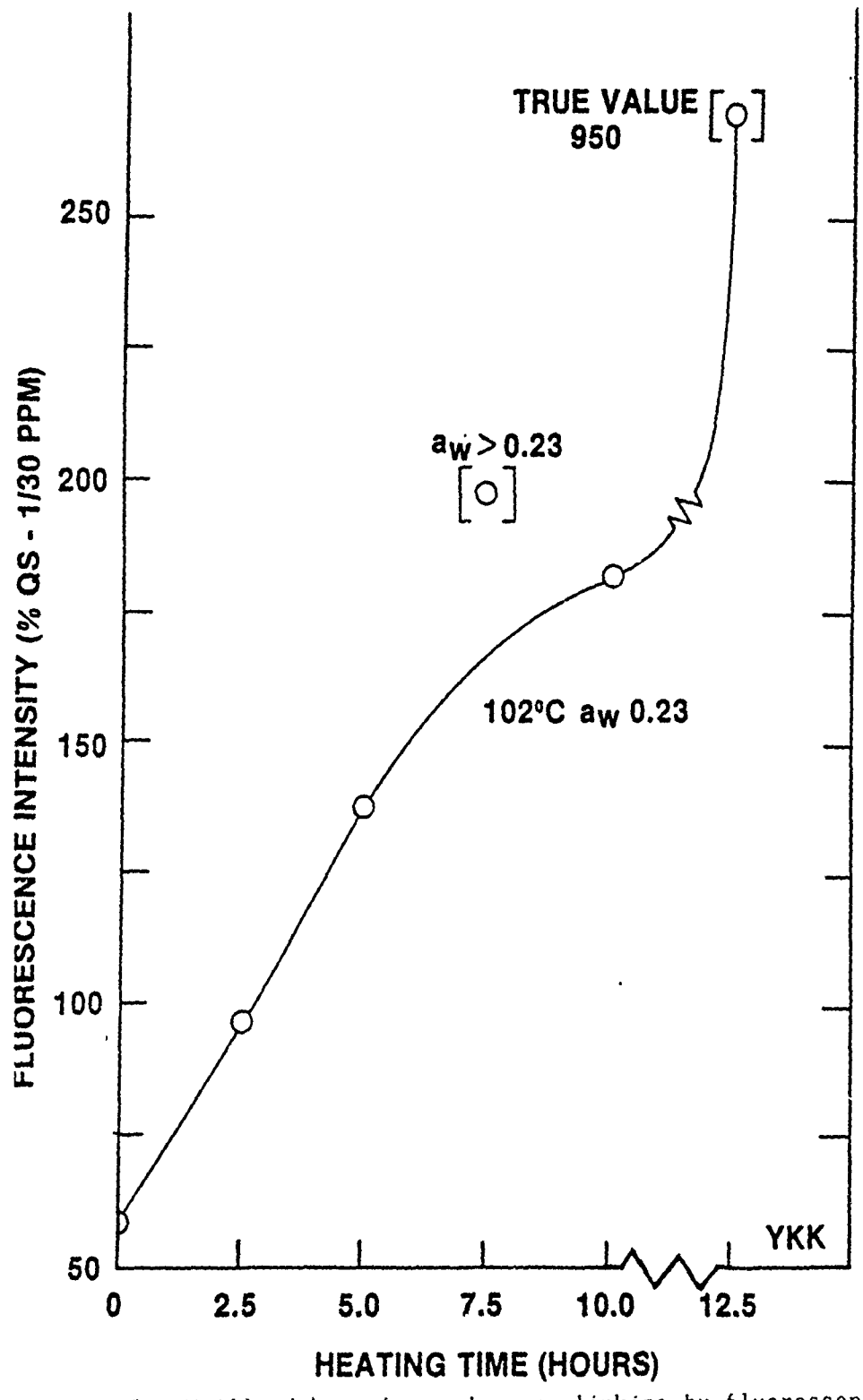

Figure 13. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 102°C, Aw 0.23. Y.K. Repeat

TABLE 3. Fluorescence of Chloroform-Methanol Extract and Reflectance Color Values. Encapsulated Energy-Dense Model Syustem Stored at 100°C Under Maillard Versus Oxidative Conditions.

SAMPLE	CONDITIONS	TIME (H)	HUNTER[1] L	a	b	C/M[1] FLUORESCENCE	C/M(L)/100
Heavily Browned	No $CoCl_2$, $NaBr_{sat}$ TBHQ 1%	12	46.2 ± 1.21	10.4 ± 0.03	15.9 ± 1.10	630 ± 30	291
Heavily Oxidized	$CoCl_2$, Drierite No TBHQ	30	69.8 ± 1.53	5.7 ± 0.30	24.6 ± 0.25	978 ± 186	683

[1]Values are means ± mean deviation (N=2)

Figure 14 shows results from browning of a dry sample (a_w 0.23) for periods of up to 10 days at 80°C. The intense fluorescence is probably largely oxidative in origin and will be discussed below. Figure 15 records increase in fluorescence of a fluid sample browned at 100°C. The typical lag at 100°C and the relatively low level, even after 12 hours, indicates probably little oxidative contribution to the fluorescence.

3. Front-face Fluorescence of Acid-precipitated Casein Slurry.

Figure 16 shows the development of Maillard front-face fluorescence in acid-precipitated casein from a 1/1 dispersion of nonfat dry milk in water at 100°C. The similarity to Figure 15 is striking.

B. Lipid Oxidation

1. Chloroform/Methanol Extract.

As we indicated in paragraph A. 2. above, if heating is prolonged, particularly under dry conditions where Maillard browning is much reduced,

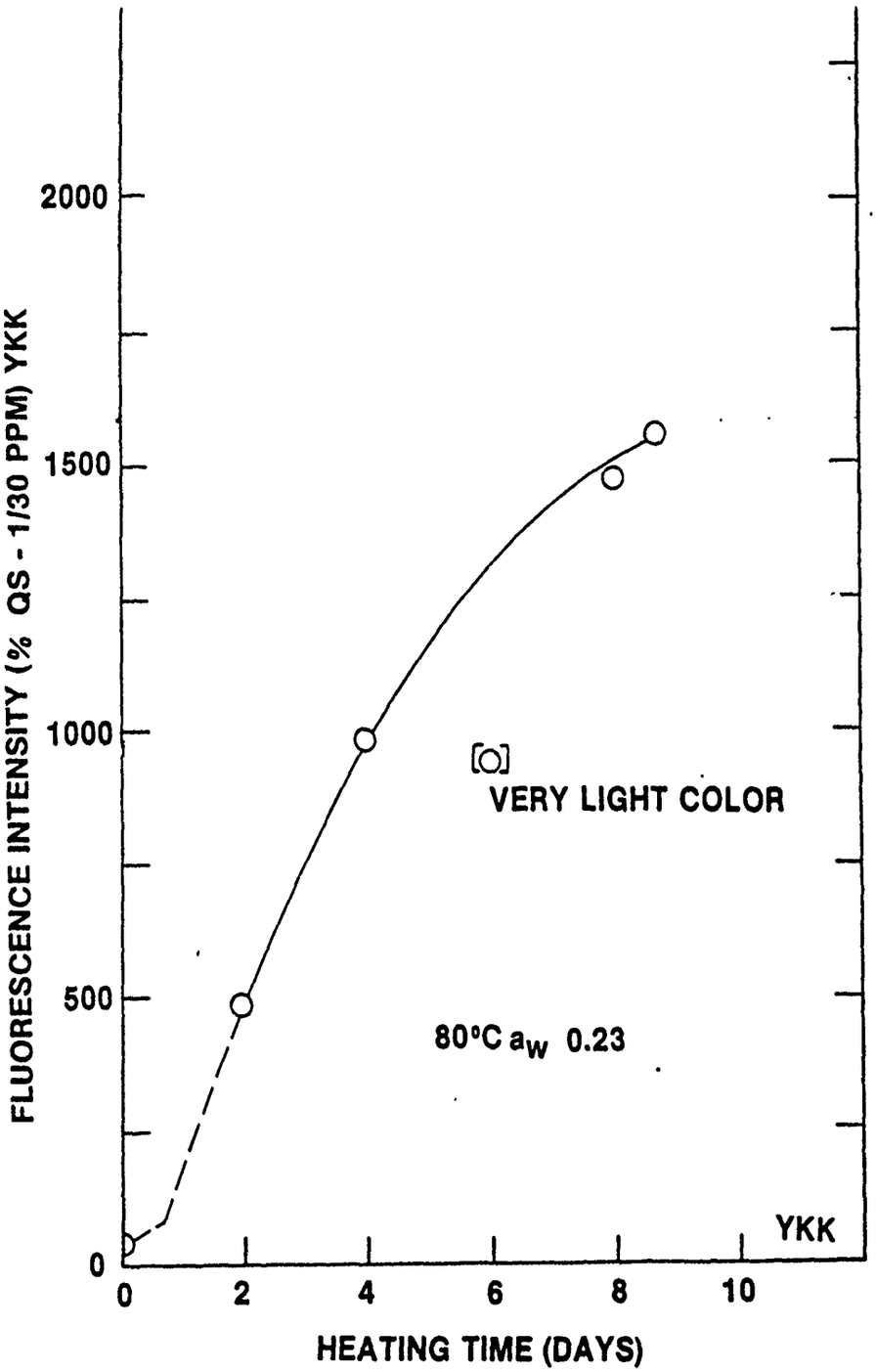

Figure 14. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 80°C, Aw 0.23. Y.K.

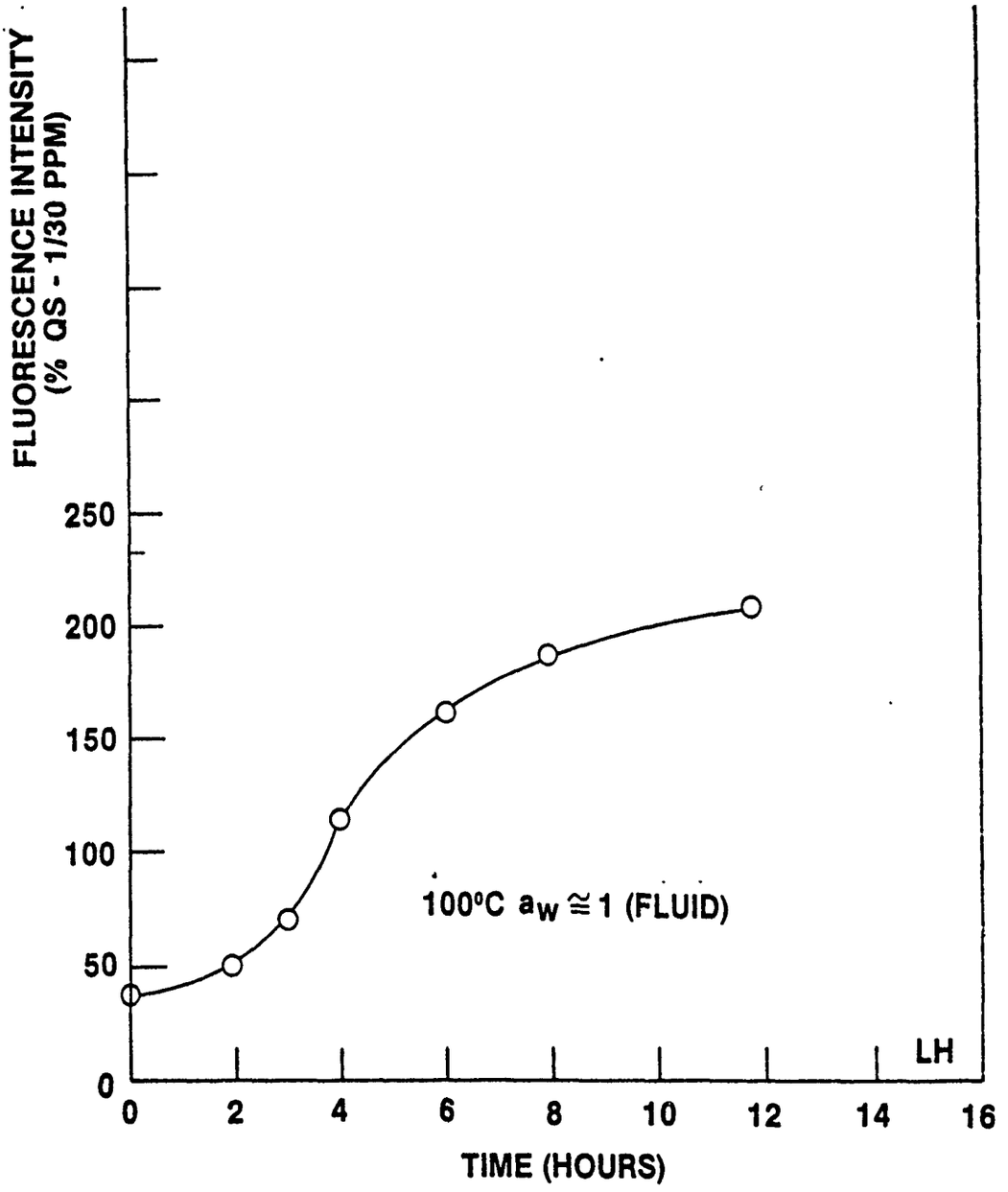

Figure 15. Maillard browning and cross-linking by fluorescence of chloroform/methanol extract. 100°C, Aw approximately 1.0 Fluid. L.H.

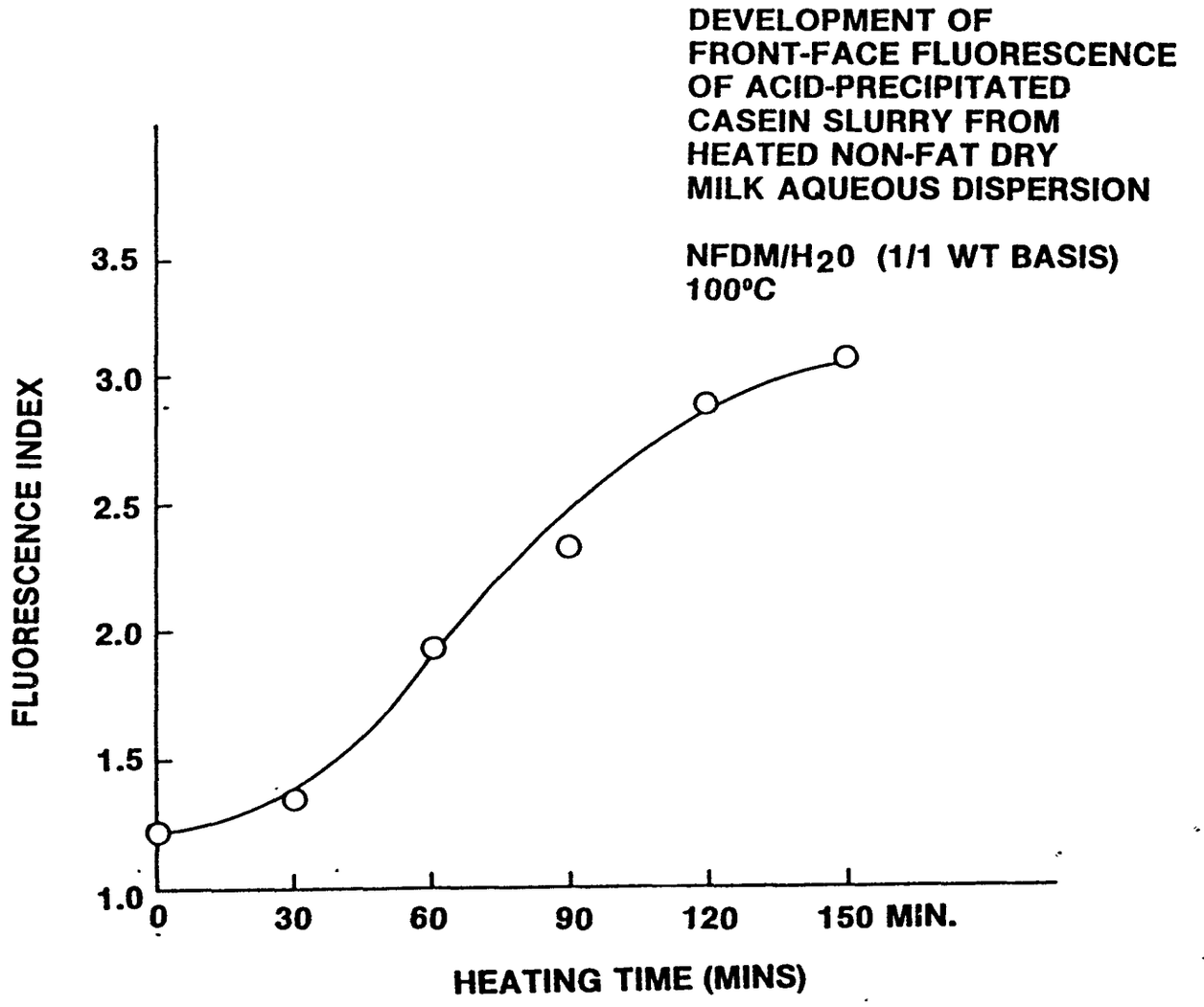

Figure 16. Development of front-face fluorescence of acid-precipitated casein slurry from heated nonfat dry milk aqueous dispersion. NFDM/H_2O (1/1 weight basis). 100°C.

an intense fluorescence supervenes at high lightness (L) as illustrated in Table 3. This is not paralleled by increase in brown color of the powder. The model system powders become rancid in odor and the vapor phase polyamide fluorescence test indicates the onset of oxidation. Figure 14 shows the increase in C/M fluorescence resulting from browning of a dry (a_w Ø.23) sample at 8Ø°C for periods of up to ten days. In the very early stages (less than one day) Maillard browning predominates, but the figure shows the onset of the intense, presumably oxidative, fluorescence, reaching very high levels at high lightness in 1Ø days of storage.[7,8,9] Work in this laboratory is underway to differentiate the oxidative from the Maillard fluorescence by excitation and emission wavelengths, synchronous scanning and 3-D depiction of "fingerprints" of wavelength families, since the spectra are complex.

2. Oxidative Polyamide Fluorescence (OPF).

Using the vapor phase detection of oxidation by means of polyamide fluorescence and plotting fluorescence index as defined above, the curves of Figure 16 were obtained for the freeze-dried model system alone and with cobaltous chloride added as pro-oxidant, both at 1ØØ°C and room temperature. The effect of the cobalt accelerator is clear, as is the accelerating effect of temperature. The room temperature sample, with cobalt, began moderate oxidation at about one day. Under these very dry conditions, the highest levels of fluorescence index are achieved. If one heats the sample at the same temperature, but at a_w Ø.23 over saturated potassium acetate (Figure 17) the rate is somewhat reduced, but again, high levels of OPF adducts are produced. When, however, as in Figure 18, water activity is at the moist level of Ø.5 (sodium bromide), the maximum

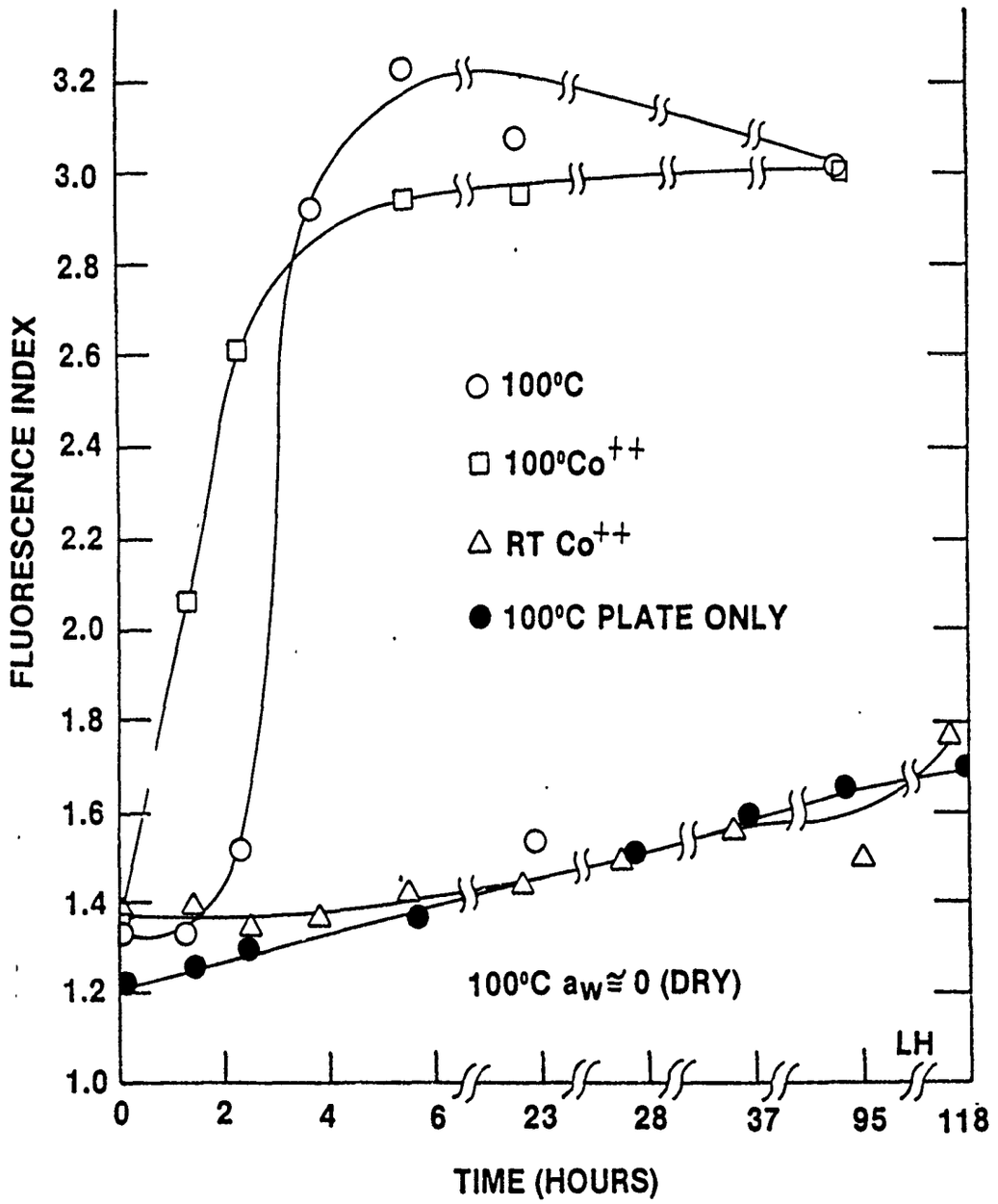

Figure 17. Polyamide fluorescence over oxidizing encapsulated energy-dense model system. 100°C, Aw approximately 0.0. L.H.

level of oxidized products is much reduced, although the time of initiation of fluorescence is little delayed. The data of Figures 17 and 18 were obtained without pre-equilibration of the samples against the specified water activity. When 24 hour pre-equilibration was done in a concurrent experiment with both levels of water activity (Figure 19) essentially the same curves were obtained. Figure 20 shows results with a fluid system, both with and without antioxidant. The oxidation rate is the lowest found and the maximum intensity of fluorescence is also low. The reproducibility of the method is shown by the two samples containing antioxidant, and the effect of antioxidant is clear, although propyl gallate is not the most suitable antioxidant for this application (Figure 21).

3. Front-face Fluorescence of Acid-precipitated Casein Slurry.

Results using this method to detect oxidation where Maillard browning is suppressed have shown it to be very sensitive. As in the chloroform-methanol extract, high fluorescence intensity at high color lightness is diagnostic of oxidation.

CONCLUSIONS

The purpose of this work was to develop methods suitable for rapid, reproducible assessment of polymerization due either to sugar-amine browning or lipid oxidation in energy-dense, encapsulated model ration systems, so that shelf life prediction and preventive measures could be developed. It is concluded that both fluorescence and near ultraviolet absorption due to compounds formed between primary amine groups and products of the degradation processes are available for the purpose.

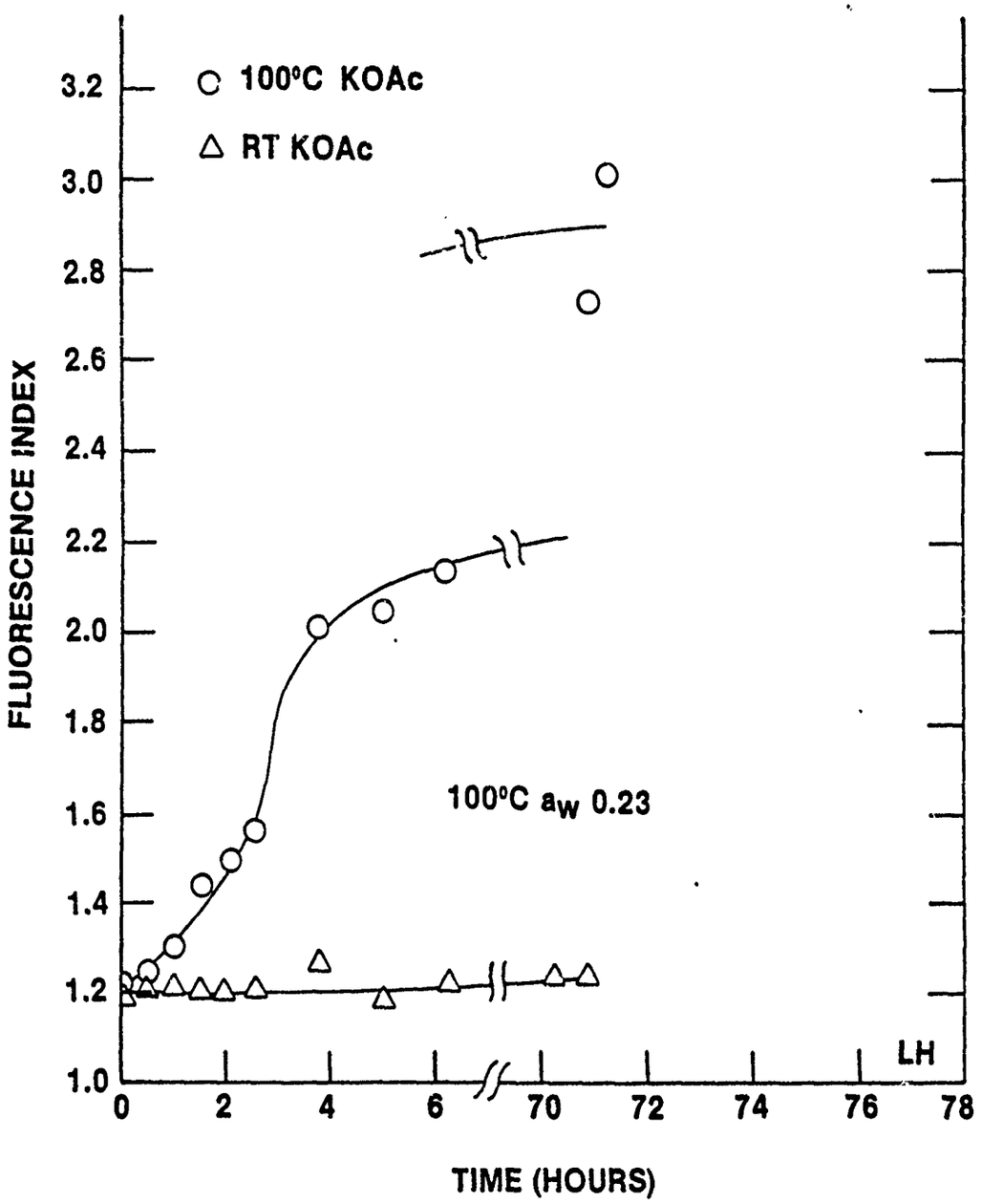

Figure 18. Polyamide fluorescence over oxidizing encapsulated energy-dense model system. 100°C, Aw 0.23. L.H.

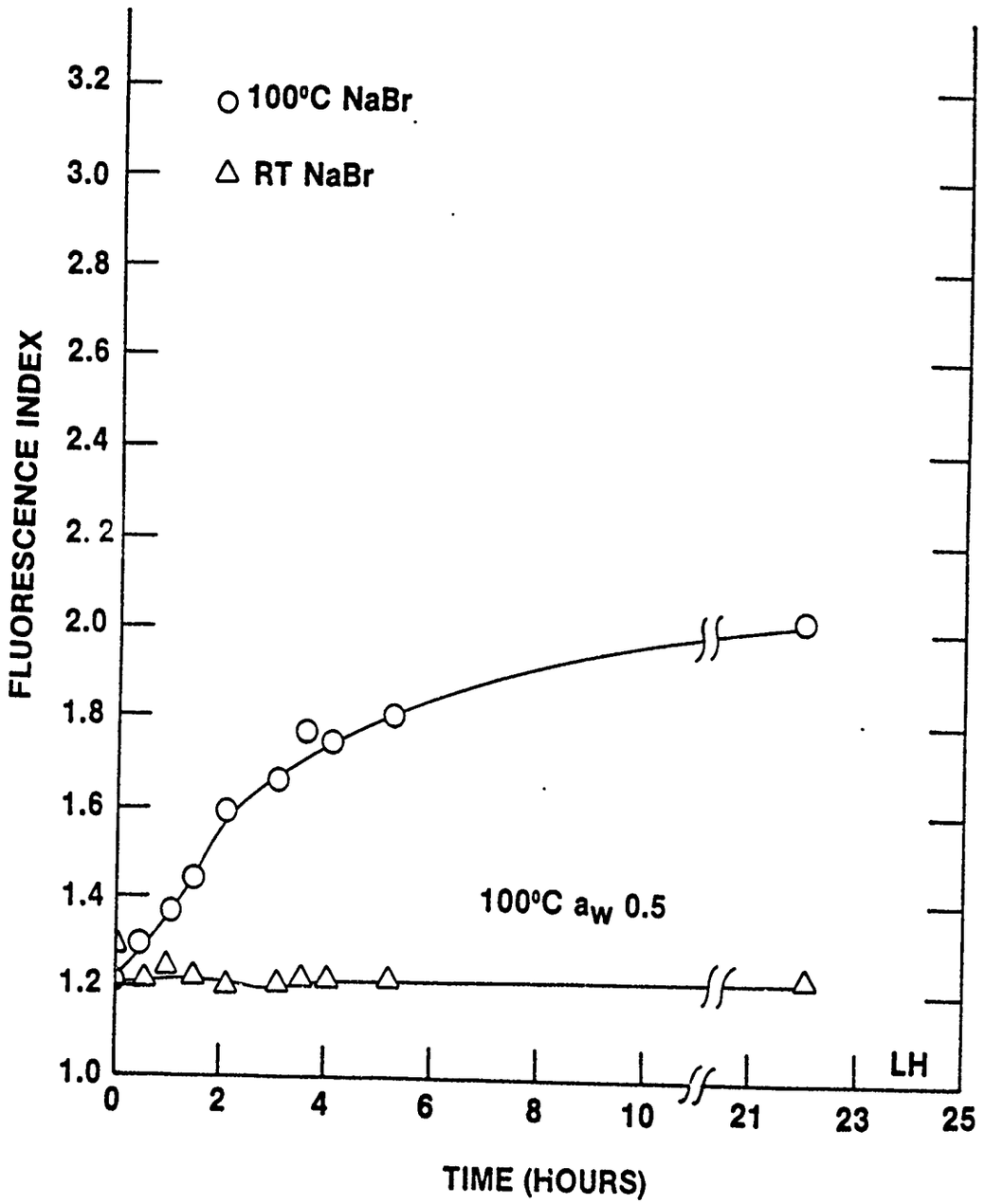

Figure 19. Polyamide fluorescence over oxidizing encapsulated energy-dense model system. 100°C, Aw 0.5. L.H.

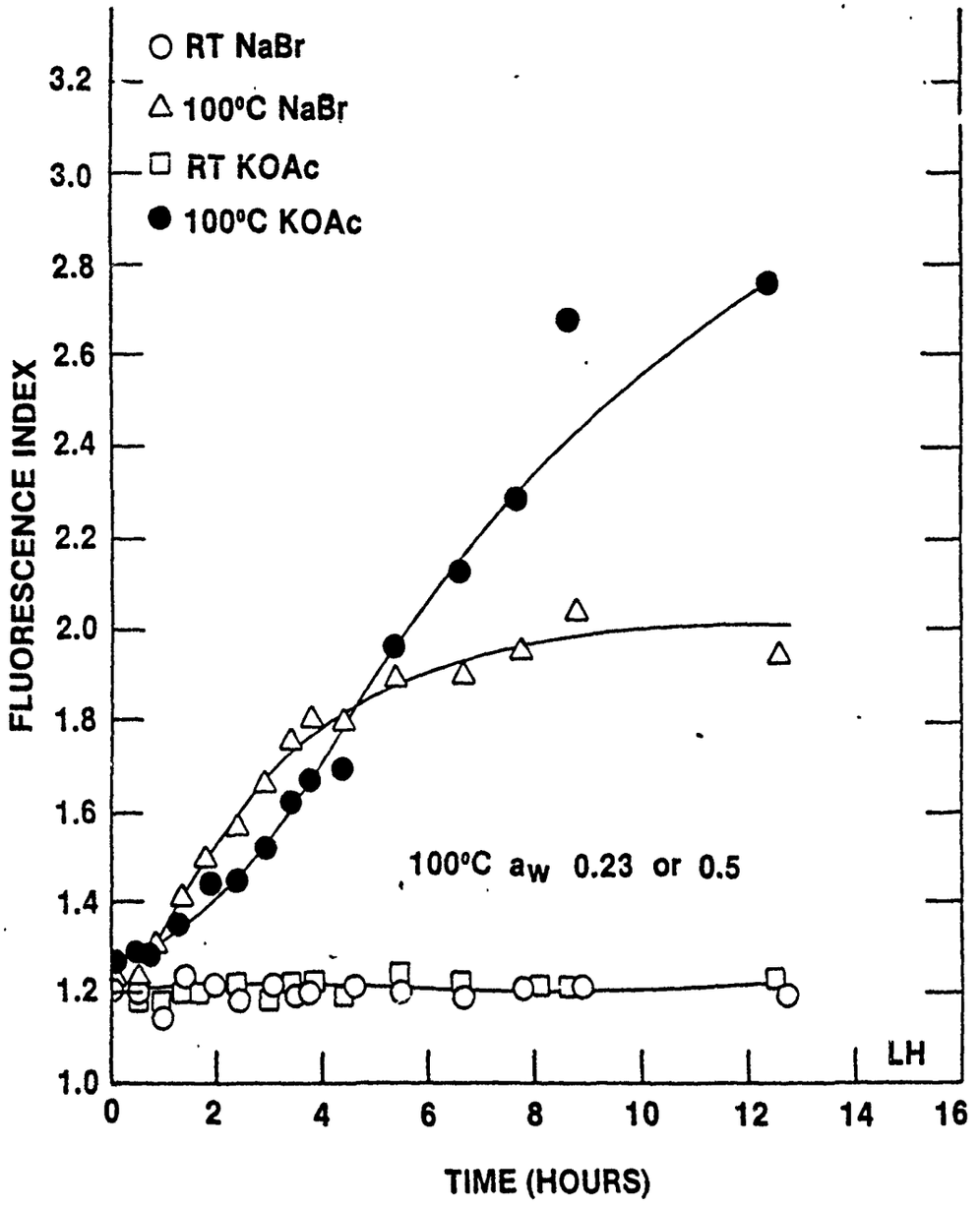

Figure 20. Polyamide fluorescence over oxidizing encapsulated energy-dense model system. 100°C, Aw 0.23 and 0.5. L.H.

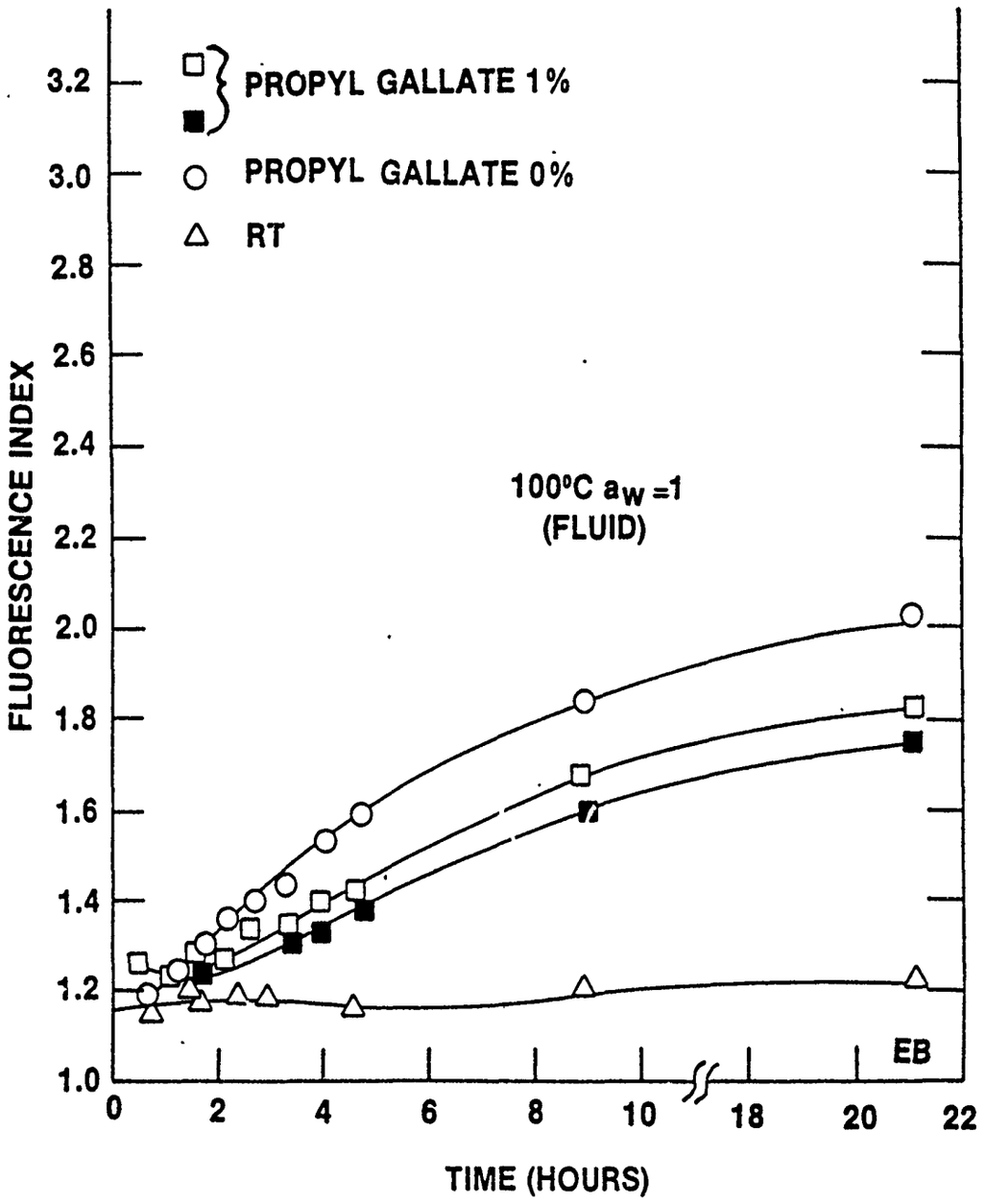

Figure 21. Polyamide fluorescence over oxidizing encapsulated energy-dense model system. 100°C, Aw approximately 1.0 Fluid. E.B.

The amine groups may arise from proteins, amino acids or amine-containing phospholipids and the complexing carbonyl groups from reducing sugars or aldehydes resulting as secondary products from oxidation, like malondialdehyde, enals or dienals.

For sugar-amine (Maillard) browning, enzymatic digest methods give high signal-to-noise results, but are time- and labor- consuming. Cold acid-precipitated casein slurries give a rapid, but less intense and slightly less reproducible signal. Automated methods based on chloroform-methanol extracts of polymerized, fluorescing phospholipids and front-face fluorescence of slurries of the residual protein are the most promising for rapid, reproducible results, and are recommended for use in most food systems, since interfering compounds are usually few and can be removed simply, unlike aqueous system contaminants.

For lipid oxidation, a vapor phase method using polyamide fluorescence due to compounds arising from gas phase products of oxidizing lipids has been found most satisfactory, since the method cleanly separates oxidation from possible concurrent Maillard browning. A back-up method was found in the chloroform-methanol extract of phospholipids, which, at the onset of lipid oxidation, produces an enhanced fluorescence-to-color intensity ratio which differs sharply from the low fluorescence-to-color ratio characteristic of Maillard browning. Front-face fluorescence of slurries of the residual protein after extraction is also a sensitive detector of oxidative cross-linking, characterized by high fluorescence at high lightness (Hunter L).

REFERENCES

1. Quartermaster Research & Engineering Center, "Southwest Asia: Environment and Its Relationship to Military Activities", Technical Report EP-118, Environmental Protection Research Division, July 1959, Natick, MA.
2. L. K. Crowe, R. Jenness, and S. T. Coulter, J. Dairy Sci. 31, 595 (1948).
3. P. A. Finot, R. Deutsch, and E. Bujard, The Extent of the Maillard Reaction in the Processing of Milk, in Maillard Reactions in Food, Ed. C. Eriksson, Progress in Food and Nutrition Science, Vol. 5, 1981, p. 345.
4. D. H. Wheeler, Oil and Soap 9, 89 (1932).
5. R. O. Sinnhuber and T. C. Yu, Food Tech. 12, 9 (1958).
6. Kim, H.-J. and I. A. Taub, J. Food Sci. 49, 699 (1984).
7. Kamerei, A. R. and M. Karel, J. Food Sci. 49, 1517 (1984).
8. Bouzas, J., A. R. Kamerei and M. Karel, J. Food Processing and Preservation 9, 11 (1985).
9. Bouzas, J., A. R. Kamerei and M. Karel, J. Food Sci. 50, 1515 (1985).
10. W. L. Porter, E. D. Black, A. M. Drolet, and J. G. Kapsalis, Analytical Use of Fluorescence-Producing Reactions of Lipid- and Carbohydrate-Derived Carbonyl Groups with Amine End Groups of Polyamide Powder. In, The Maillard Reaction in Foods and Nutrition, Eds. G. R. Waller and M. S. Feather, ACS Symposium Series No. 215, American Chemical Society, Washington, D.C., 1983, p. 47.
11. K. S. Chio and A. L. Tappel, Biochem. 8, 2821 (1969).